FUTURE APPLICATIONS OF NEAR INFRARED SPECTROSCOPY (NIRS) IN THE SOUTH AFRICAN BRANDY AND DISTILLATION INDUSTRY

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature:

Date:

ABSTRACT

This study shows the potential of near infrared spectroscopy for both qualitative and quantitative analyses of wine and brandy in the distilling industry.

Wines intended for further processing by the distillation industry have to meet certain specifications to ensure an efficient and cost-effective distilling process with a suitable product yield. Certain compounds have, therefore, been recognised as significant quality parameters and through their regulation, a product of consistent quality can be ensured. These quality parameters include alcohol, total acid, volatile acid, total sulphur dioxide, total phenolics, reducing sugars and acetaldehyde concentrations and pH. The effective control of all these compounds in the wines prior to distillation, is critical to maintain the feasibility and efficiency of the process.

Fourier transform near infrared spectroscopy (FT-NIRS) can be used as a rapid and accurate analytical method for the determination of alcohol concentrations in distilling and brandy base wine. The choice of path length for the liquid sampling cell is an important consideration. In this study, alcohol with its strong absorption of OH bands in the near infrared region was not significantly affected, but difference in path length showed a significant influence on the prediction of the volatile acid concentration in distilling wine. Very strong correlations were found between the spectral data and the alcohol concentration data in distilling wine ($r = 0.99$, SEP = 0.18% v/v, RER =30) and brandy base wine ($r = 0.92$, SEP = 0.18% v/v, RER = 11.1). Good predictions were obtained for the volatile acid content of brandy base wine ($r = 0.85$, SEP = 0.04 $g.L^{-1}$) but better accuracy could be obtained by incorporating a wider concentration range and more sensitive, although not the accepted reference methods, such as HPLC and GC, into the modelling. In contrast to the brandy base wine, the much cruder, turbid distilling wine ($r = 0.67$, SEP = 0.33 $g.L^{-1}$) yielded poor calibration results for volatile acid.

FT-NIRS can also be used as a rapid screening method to measure the total acid content, pH and total phenol levels in brandy base wine samples. Satisfactory predictions were obtained for the total acid content ($r = 0.89$, SECV = 0.38 $g.L^{-1}$), pH ($r = 0.84$, SEP = 0.09) and total phenol levels

($r = 0.71$, SEP = 16.4 $mg.L^{-1}$ GAE). The SEP of all the parameters compared well with the SEL and were within acceptable limits. The range of the concentration measurements were, however, very narrow and the accuracy of these models (measured as the ratio of the range to the standard error of prediction) were all below 10, indicating that modelling of these parameters in brandy base wine should be attempted with reference values covering a wider range. Poor correlations and predictions were obtained between the wine spectral data and the acetaldehyde ($r = 0.39$, SECV =1.45 $mg.L^{-1}$), residual sugar ($r = 0.58$, SECV =0.49 $mg.L^{-1}$) and total sulphur dioxide ($r = 0.35$, SEP = 10.9 $mg.L^{-1}$) concentration data.

The unique character of potstill distillates, originating from the grape cultivar, type and age of the oak casks, lends itself to the development and blending of different brandies. The process of blending and correcting the brandy is necessary to obtain a product and brand of established and recognised character. The brandy is generally divided into different classes prior to blending based on the degree of "smoothness" or "hardness" of the matured distillate as determined by a sensorial panel. The ideal would, however, be to replace this subjective evaluation method with a more objective approach, such as an instrumental method like NIRS.

Near infrared spectroscopy showed potential to successfully discriminate and classify brandy samples in terms of their sensorial classification status. The near infrared spectra of unblended three-year old brandies were measured and discriminant analysis performed using the subjective sensory classification data of the brandy. SIMCA (soft independent modelling of class analogy) models were developed by calculating separate PCA (principal component analysis) models for each of the classes for which qualitative analysis were required. An assessment of class membership of an unknown sample was made on the basis of the distance to the cluster centroid. Two types of NIRS instruments, a Fourier transform near infrared system and a diode array spectrophotometer were employed to examine the discriminatory abilities of NIRS. Excellent predictions (90.9%-100%) were obtained for the "smoothest" and "hardest" classes within the 2000 and 2001 seasons with FT-NIRS. For the diode array spectra reasonable classification data were obtained for all the classes within the 2000 and 2001 seasons, as

well as an excellent classification rate (100%) of the smoothest class in the 1999 season. Prediction results for class membership of the brandy samples from the three season's combined data did not deliver satisfactory discriminatory results. By applying NIRS and SIMCA, rapid and objective classification of brandy samples within seasonal context can be established to enhance the blending processes.

Having accurate reference data in the calibration set is essential in near infrared spectroscopy (NIRS). The precision (usually decided by blind duplicate determination) of the reference data serves as the performance target for correlation-based near infrared analysis. The differences between reference values obtained from two independent laboratories and the Foss Winescan FT 120, which is also a predictive method, for the alcohol and volatile acid determinations in distilling wine and between two independent laboratories for the measurement of the total sulphur dioxide content, were investigated. ANOVA and post-hoc analysis (Bonferroni testing) revealed statistically significant differences ($p<0.05$) between all three measurements for the volatile acid analysis. One laboratory differed significantly from the other two measurements in its measurement of the alcohol content in distilling wine. A paired t-test performed on the two laboratories' values obtained for total sulphur dioxide revealed statistically significant differences ($p<0.05$) between the two sets of results. This study restated the importance of obtaining reliable reference data for calibration purposes to maintain the efficiency and accuracy and therefore the reputability of NIRS as an accurate and reliable analytical method.

UITTREKSEL

Hierdie studie dui op die potensiaal van naby infrarooi spektroskopie (NIRS) vir beide die kwalitatiewe en kwantitatiewe analise van wyn en brandewyn in die distillasie industrie.

Sekere spesifikasies word gestel waaraan wyn met voldoen voor dit verdere prosessering tydens distillasie kan ondergaan. Die regulering van wynkwaliteit is noodsaaklik om die distilleringsproses ekonomies volhoubaar te maak. Sekere komponente in die wyn het 'n direkte invloed op die kwaliteit van die eindproduk en word beskou as betekenisvolle parameters wat beheer moet word om 'n produk van konstante hoë kwaliteit te lewer.

Hierdie parameters sluit alkohol, titreerbare suur- en vlugtige suurinhoud, asook die pH, totale swaweldioksied, totale fenol vlakke, reduserende suikers en asetaldehied konsentrasies in. Die regulering van al hierdie komponente in die wyne is essensieel voor die aanvang van distillasie om die effektiwiteit en produktiwiteit van die proses te verseker.

Fourier transformasie naby-infrarooi spektroskopie (FT-NIRS) kan benut word as 'n vinnige en akkurate analitiese metode om die alkohol konsentrasie in stook- en rabatwyn te bepaal. 'n Geskikte padlengte kuvet moet gekies word vir die ontleding van vloeistof monsters met NIRS. In hierdie studie is gevind dat die voorspelling van alkoholkonsentrasie in wyn, nie soseer beïnvloed is deur die keuse van padlengte nie. 'n Vlugtige komponent soos asynsuur, wat die maatstaf van die vlugtige suurinhoud van die wyn is, word egter regstreeks beïnvloed deur die keuse van padlengte. 'n Sterk korrelasie is gevind in die datastel vir die alkoholinhoud van die stookwyn ($r = 0.99$, SEP = 0.18% v/v, RER = 30) en rabatwyn ($r = 0.92$, SECV = 0.18% v/v, RER = 11.1). Baie goeie voorspellings is ook gevind vir die vlugtige suurinhoud van rabatwyn ($r = 0.85$, SEP = 0.04 $g.L^{-1}$). Die akkuraatheid van die metode (uitgedruk as die RER waarde) het egter aangedui dat 'n wyer konsentrasie omvang en meer akkurate verwysingswaardes gebruik moet word om 'n geskikte model vir vlugtige suur kwantifikasie in rabatwyn te bewerkstellig. In teenstelling met die resultate verkry vir die rabatwyn, het die ruwer, turbiede stookwyn swak gekalibreer vir die vlugtige suurinhoud ($r = 0.67$, SEP = 0.33 $g.L^{-1}$).

FT-NIRS kan ook gebruik word as 'n vinnige seleksie metode om titreerbare suur vlakke, pH en totale fenolinhoud van rabatwyn monsters te bepaal. Sterk korrelasies is in die datastel gevind vir die titreerbare suurinhoud (r = 0.89, SECV = 0.38 $g.L^{-1}$), pH (r = 0.84, SEP = 0.09) en totale fenol vlakke (r = 0.71, SEP = 16.4 $mg.L^{-1}$ GAE) van rabatwyn. Die SEP waardes verkry vanaf die NIRS bepalings het baie goed vergelyk met die standaard foute wat verkry is vir die chemiese bepalings. Die konsentrasie omvang van die verwysingswaardes was, egter, beperk en die akkuraatheid van die NIRS modelle (uitgedruk as die verhouding tussen die konsentrasie omvang en die standaard fout van voorspelling) was laer as 10. Dit het daarop gedui dat die NIRS kalibrasie van die parameters eerder uitgevoer moet word met monsters wat 'n wyer konsentrasie omvang dek en gemeet is met meer sensitiewe analitiese metodes soos HPLC en GC. Die kalibrasie en voorspelling van die asetaldehied inhoud (r = 0.39 , SECV =1.45 $mg.L^{-1}$), reduserende suiker konsentrasie (r = 0.58, SECV =0.49 $mg.L^{-1}$) en totale swaweldioksiedinhoud (r = 0.35, SEP = 10.9 $mg.L^{-1}$) het onaanvaarbare voorspellings gelewer.

Die unieke karakter van potketel distillate wat bepaal word deur die druifkultivar, tipe en ouderdom van die eikehoutvate, bied die potensiaal vir die ontwikkeling en versnyding van verskillende style brandewyn. In 'n poging om 'n produk en handelsnaam van erkende en gevestigde karakter te verkry, moet die brandewyn eers versny word na veroudering. Die brandewyn word geklassifiseer in verskillende style op grond van sensoriese gehalte. NIRS is gebruik as 'n vinnige tegniek om te onderskei tussen verskillende style onversnyde drie jaar-oue brandewyn.

SIMCA *(soft independent modelling of class analogy)* modelle is ontwerp deur aparte PCA (hoof komponent analise) modelle vir elke klas waarvoor kwalitatiewe analise vereis is, te ontwikkel. 'n Skating van klas lidmaatskap van 'n onbekende monster is gemaak deur die afstand van die monster na die groepsmiddelpunt te bereken.

Twee soorte NIRS instrumente, 'n FT-NIR en 'n *diode array* spektrofotometer is gebruik tydens die studie om die diskrimineringsvermoë van elk te ondersoek. Uitstekende voorspellings (90.9%-100%) is gekry vir die "sagste" en "hardste" klasse binne die 2000 en 2001 seisoene met FT-

NIRS. Met die *diode array* spektra is redelike klassifikasie resultate verkry vir al die klasse binne die 2000 en 2001 seisoene, asook 'n uitstekende klassifikasie (100%) vir die sagste klas binne die 1999 seisoen. Voorspellings vir klas lidmaatskap van die brandewyn monsters vir die drie seisoene se gekombineerde data het egter onaanvaarbare diskriminasie resultate gelewer. NIRS en SIMCA sal 'n vinnige en objektiewe klassifikasie proses van onversnyde brandewyn monsters kan meebring wat tot voordeel van die versnydings- en produksieproses sal wees. Hierdie voorspellings sal egter binne seisoensverband moet geskied.

Akkurate verwysingsdata in die kalibrasie datastel is 'n essensiële fasset van naby infrarooi spektroskopie. Die presisie (bepaal deur blinde duplikaat bepalngs) van die verwysingsdata dien as 'n prestasie teiken vir korrelasie-gebaseerde naby infrarooi voorspellings. Die verskille tussen die data verkry vir die alkohol- en vlugtige suurinhoud van stookwyn deur twee onafhanklike laboratoriums en 'n instrumentele metode (Foss Winescan FT 120) asook die verskille tussen die data verkry van twee laboratoriums vir die totale swaweldioksied metings, is ondersoek. ANOVA en post-hoc toetse het aangedui dat al die data verkry vir die vlugtige suur bepalings, statisties betekenisvol van mekaar verskil het ($p<0.05$). Vir die alkohol bepalings in stookwyn, het die data verkry van een laboratorium betekensvol verskil van die ander twee datastelle. Gepaarde t-toetse uitgevoer op die data verkry vir totale swaweldioksiedinhoud, het ook betekinisvolle verskille aangedui tussen die twee laboratoriums. Hierdie studie het die belangrikheid van betroubare verwysingsdata vir kalibrasie doeleindes beklemtoon, veral waar 'n tegniek soos NIRS se werksverrigting afhang van die akkuraatheid van dié waardes.

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Wilhelm. As jy eendag in die hemel kom wag daar 'n ekstra paar vlerkies vir jou!

Dedicated to my parents, Philip and June

"Well, I sort of made it up", said Pooh...
"It comes to me sometimes."
"Ah", said Rabbit, who never let things come to him,
but always went and fetched them.

A.A. Milne. (House at Pooh Corner)

CONTENTS

Language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science and Technology.* This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

CHAPTER 1

INTRODUCTION

CHAPTER 1

INTRODUCTION

"There is brandy in this bottle, dominee', Gert Bekker said to the predikant, 'that I keep for the sake of my horses on cold nights, like now. It is an old Marico remedy for when horses are in danger of getting the floute, I take a few mouthfuls of the brandy, which I then blow into the nostrils of the horses, who don't feel the cold so much, after that. The brandy revives them."

Herman Charles Bosman.

Brandy is an unique alcoholic beverage produced through the distillation of brandy base (rebate) wine followed by a maturation process performed in oak barrels. Basically, the composition of brandy consists of ethanol and water with a variety of congeners or congenerics that contribute to the unique aroma, flavour and colour of the final product (Guymon, 1973; Weitz, 2001). New oak casks are used for the maturation of fresh distillates and the emerging products show considerable variation in terms of character, flavour and depth of colour (Anonymous, 1988). Blending is performed by adding and correcting levels of constituents to obtain both uniformity within a brand name, and a final product of established and recognised character. Lower cost brandies are subjected to sensory evaluation by a trained panel prior to blending it into commercial brands. Different distilleries employ different blending methods, but generally the unblended brandy is divided into different classes according to taste, softness, fullness and flavour intensity. The brandy is then blended into a style most suited to the character of the specific class (Steger, 2001; Weitz, 2001). A rapid automated method that could objectively discriminate between the different classes of brandy would enhance the production process of lower cost commercial brandies. This would not only streamline the process, but also result in a more consistent classification procedure and possibly a more consistent product.

The compositional quality of the wine used as base products during distillation, is of considerable significance as the grape composition contributes to the unique character of the final spirit (Strauss & Williams, 1983). The secondary products of fermentation (alcohols, esters, volatile acids) have a very significant influence on the

aroma of the young wines and these are carried over during the distillation process (Bertrand, 1983).

Brandy base wines are produced in all the major wine-producing areas of South Africa, including the winelands of the Western and Southern Cape, as well as the grape producing areas surrounding the fertile Orange River (SAWIS, 2002). In 1997, 160 million litres of brandy base wine with a value of nearly 200 million rand were sold to wholesalers locally. The composition of the base wine is regulated legally and rapid and reliable methods of analyses are therefore critical to guarantee the quality of the end product. The parameters that are indicative of the quality of brandy base wine and which have to be regulated are alcohol, total acid, volatile acid, total sulphur dioxide, total phenols and residual sugar content plus pH (Steger, 2001). Acetaldehyde contributes significantly to the flavour profile of the finished product and controlling the levels could improve the quality of the brandy. Near infrared spectroscopy could be used to do simultaneous analyses of all the quality parameters on the wine samples.

Distilling wine is used in the distillation process to obtain alcoholic grape spirit, which in turn is used in the production of an array of alcoholic beverages, including blending of brandy. The production of distilling wine exceeds 130 million litres annually in South Africa and is an important source of income for many grape and wine producers (SAWIS, 2002). The distilling wine is fermented from lower quality grapes and pressed juice that are not suitable for the production of table wine or brandy base wine (Steger, 2001). Distilling wine may be regarded as a wine of inferior quality, but it yields alcohol, which has a great economical value. The compositional quality must, therefore, be regulated to prevent production and economic losses. The most important parameters to consider are alcohol content, volatile acidity and total sulphur dioxide content of the wine (Steger, 2001). NIRS could also be employed to perform simultaneous analyses of all the quality parameters on the distilling wine samples.

Throughout the history of wine production analytical techniques have become increasingly important with the development of technology and increased governmental regulation. Most of these methods are, however, time-consuming and rely on the use of various consumable chemicals, which make them rather expensive to perform and hazardous to the environment. The element of human error could also possibly lower the accuracy of the results. The distilling industry is in need of a

method to replace the present time-consuming analytical methods used. One of the major limitations of NIRS in food analyses, however, concerns its dependence on less precise and equally empirical chemical methods of analysis, and attention should be given to the precision and accuracy of the reference methods used (Osborne *et al.*, 1993).

In recent years NIRS has evolved into many specialised fields and the wine industry has kept up with the trend (Kaffka & Norris, 1976; Baumgarten, 1987; Burns, 1995; Garcia-Jares & Médina, 1997; Van den Berg *et al.,* 1997; Gishen & Dambergs, 1998; Cope, 2000; Dambergs *et al.,* 2001; Manley *et al.,* 2001). The expansion of the applications of near-infrared spectroscopy in the distilling (and wine) industry shows the potential to contribute to the optimisation of the processes involved and the improvement of quality control. The advantages of NIRS include the speed of analysis, simplicity of sample preparation, multiplicity of analyses, and non-destructive nature of the analysis.

The objectives of this study were to:

- develop rapid near infrared spectroscopic methods for the prediction of the most important quality parameters of distilling wine and evaluate the influence of path length on the NIR calibration performance;
- develop rapid near infrared spectroscopic methods for the prediction of the most important quality parameters of brandy base wine;
- investigate the potential of near infrared spectroscopy and soft independent modelling of class analogy (SIMCA) models to discriminate between different classes of unblended three-year old brandy;
- investigate the performance of two different types of NIRS equipment (Fourier transform en diode array) in their ability to discriminate between different classes of unblended three-year old brandy;
- to investigate the differences found between the corresponding reference values for the ethanol, volatile acid and total sulphur dioxide contents of distilling wine obtained by independent laboratories.

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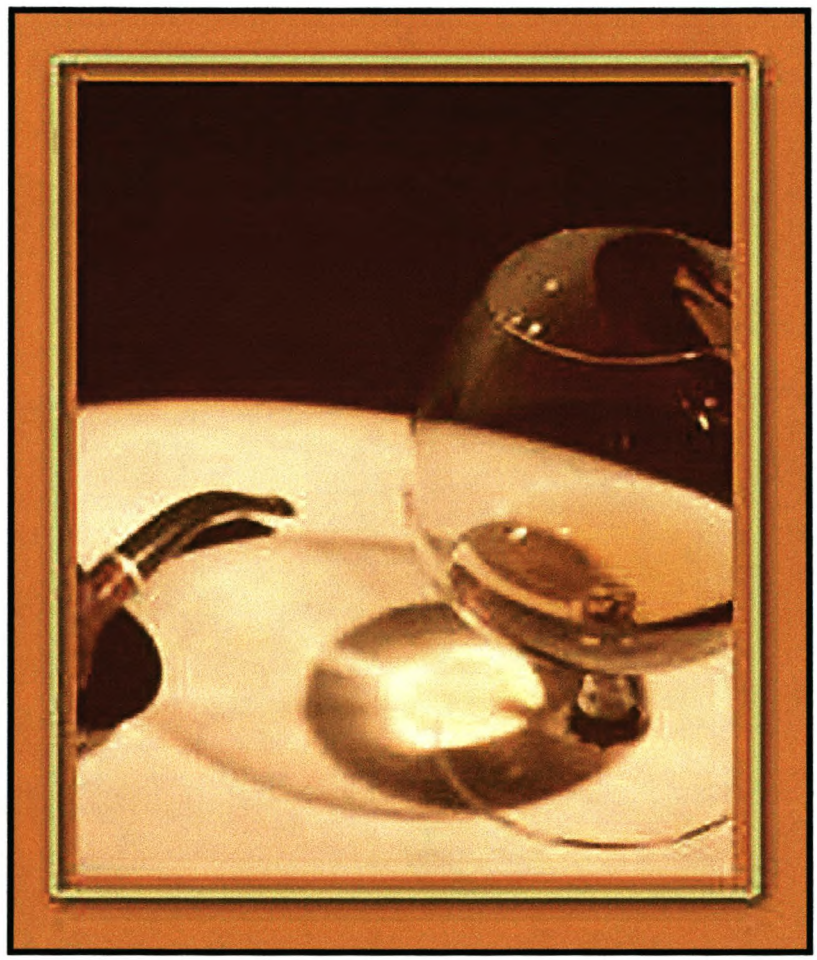

CHAPTER 2

LITERATURE REVIEW

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CHAPTER 2

LITERATURE REVIEW

"Near infrared analysis like the bee should not work. The aerodynamics of a bee are not suited to flying, but it flies anyway"

Thomas Hirschfeld.

A. Introduction to the distilling industry in South Africa

A1. The wine and alcoholic beverage industry

The South African wine industry cultivates almost 106 000 hectares of land and employs a total of 4501 primary wine producers (SAWIS, 2002). With an estimated investment value of just under R9000 million rand, the industry provides a livelihood to almost 350 000 people. The annual harvest of more than 1 million tons of grapes (including white, red and table varieties) consists on average (calculated over the period 1995 to 2000) of 65% for the production of table wine, 10% for brandy base wine (used for brandy distillation), 15% for distilling wine (used for grape spirit distillation) and the rest (10%) for non-alcoholic use (Figure 1).

South Africa can claim ownership of less than 1.4% of the worlds' vineyards, but the annual output of one billion litres makes the South African wine industry the worlds' seventh-largest, contributing 3.2% of the global wine crop (SAWIS, 2002). In 2000, 24.8 million litres of brandy base wine, with a gross value of 34 million rand, were produced for the production of brandy. The production of distilling wine exceeds 130 million litres annually (SAWIS, 2002). Brandy sales in 2000 were estimated at about 15.7 million litres and represented 6% of the alcoholic beverage market (Figure 2) and 47% (based on alcohol content) of all South African spirits sales (SAWIS, 2002).

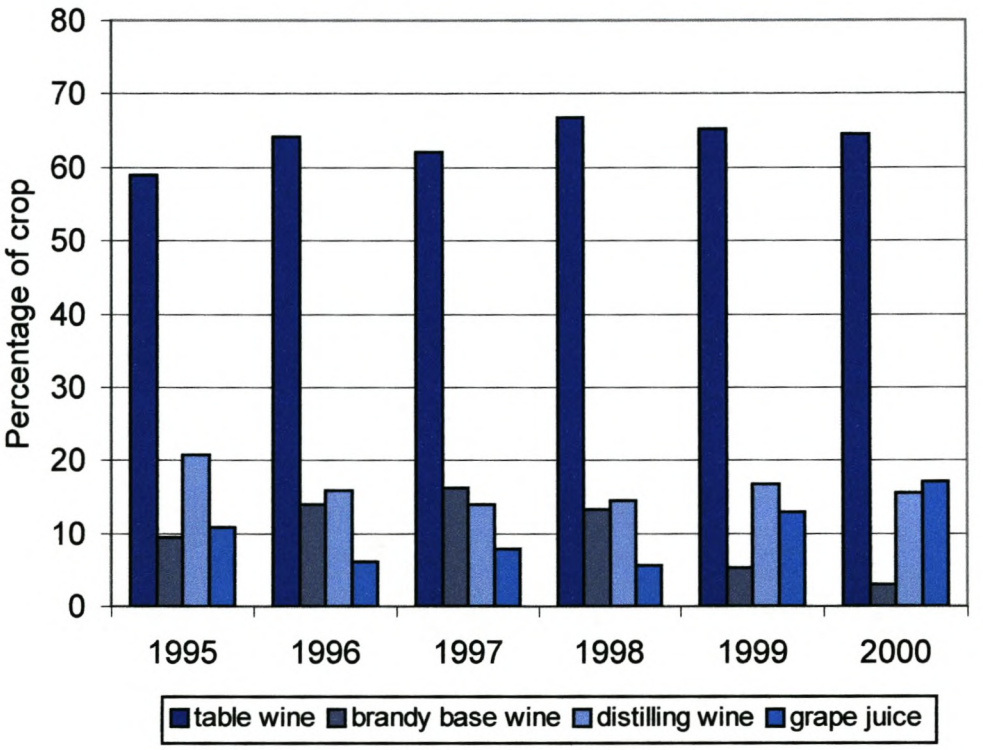


Figure 1. The percentage contributed by each sector of the grape and wine industry to the annual wine production in South Africa over the period 1995-2000 (SAWIS, 2002).

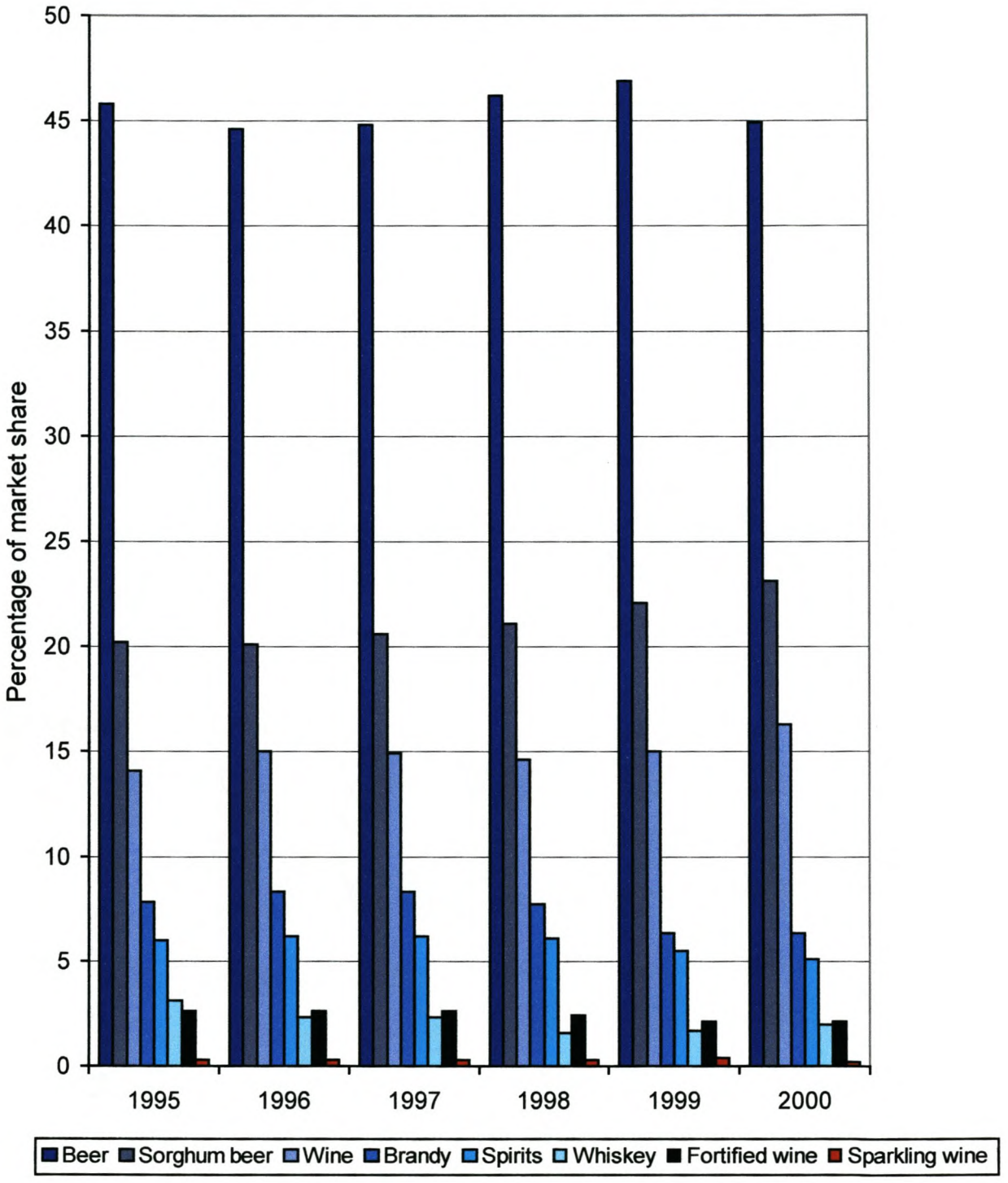


Figure 2. The market share of the different alcoholic beverages in South Africa over the period 1995 to 2000 (SAWIS, 2002).

A2. A historic overview of brandy production

The history of brandy distilling in South Africa is almost as old as that of winemaking, dating back to 1672 when a cook from the Dutch East Indian Company distilled the first brandy in the Cape (Brink, 1973). At the time the free run juice was used for wine production while brandy was crudely distilled from the skins or husks, stalks and residue from the bottom of the wine barrel in open copper pots. The distillate underwent no maturation and had a bleak watery colour. It was commonly referred to as Cape Smoke and Witblits (white lightning), attributed to the burning sensation it left in the mouth (Brink, 1973; Anonymous, 1988).

In the early 1800's, the economy in South Africa began to grow and with it came an improvement in the standard of living and a demand for better quality and luxury items (Brink, 1973). The wealthy were used to imported French Cognacs and other European brandies, which were to set the standards for South African brandies. In the years following, the well-known names of Collisons Brandy Company, Van Ryns, E.K. Green, Sedgewick and Paarlse Wijn and Brandewijn Maatschappij Beperk amongst others were established. A flamboyant immigrant Frenchman, Renè Santhagens, probably made the largest contribution to the improvement in the quality of Cape brandy. He practised the cognac method of brandy distillation, maturing it in imported casks of French oak. It was Santhagens' principles for wine selection, pot-still distillation and maturation that formed the foundation of legislation in 1909 (Brink, 1973; Anonymous, 1988; Weitz, 2001).

Today, South Africa is the fifth largest brandy producer in the world (Retief, 2002). It is estimated that more than 300 000 South Africans are directly or indirectly involved with the production, marketing and selling of brandy (SAWIS, 2002).

B. An overview of the distillation process

B1. Brandy

Brandy base wine or rebate wine is a type of wine with a unique character used specifically for the distillation of brandy. It is produced from the free run juice obtained from specific non-muscat grape cultivars, including Colombar, Chenin Blanc, Ugni Blanc, Cinsaut and Palamino (Anonymous, 1988; Weitz, 2001). Skin contact with the juice must be minimised and the pips and stalks prevented from being crushed into the juice, as the tannins and other phenolic compounds in these components impart a bitter taste in the brandy. Selected yeast strains with

favourable brandy-aroma forming characteristics are inoculated into the juice and the fermentation is regulated at 15 to 18°C. No sulphur dioxide may be added to the wine at any stage and the lees may only be removed through centrifugation. Fermentation is terminated when the residual sugar content is exhausted, usually after about 7 to 14 days (Weitz, 2001).

The brandy base wine composition is specified by the South African Wine and Spirit Board and is regulated in terms of the Liquor Products Act, Act 60 of 1989. In terms of regulation 12 (2) and 12 (3) of this act:

2) Wine intended for distillation into pot-still brandy shall have -
   a) If the solid matter of that wine is 45 percent by volume or less
      - residual sugar content, expressed as invert sugar, of not more than 4 grams per litre;
      - a volatile acid content, expressed as acetic acid, of not more than 0.7 grams per litre; and
      - a total sulphur content of not more than 20 milligrams per litre; and
   b) If the solid matter of that wine is more than 45 percent by volume
      - residual sugar content, expressed as invert sugar, of not more than 4 grams per litre;
      - a volatile acid content, expressed as acetic acid, of not more than 1.2 grams per litre; and
      - a total sulphur content of not more than 70 milligrams per litre; and
3) No fining agent shall be used in the preparation of wine intended for distillation into pot-still brandy.

The wine for pot-still brandy is distilled through a double distillation process in a discontinuous fashion in copper kettles as depicted in Figure 3 (Weitz, 2001). The distillation process involves two phases of which the first involves the distillation of 9-12 percent alcohol per volume (% alc/vol) wine to crude-brandy (30% alc/vol). The second distillation process involves the distilling of the crude-brandy to pot-still brandy with an alcohol content of no more than 75% alc/vol as shown in Figure 4 (Weitz, 2001). The first distillation can be regarded as a concentrating process and can last up to 8 hours, but is usually completed within 6 to 7 hours. The volume of this product is only a third of the volume of the original bulk wine and is very rough

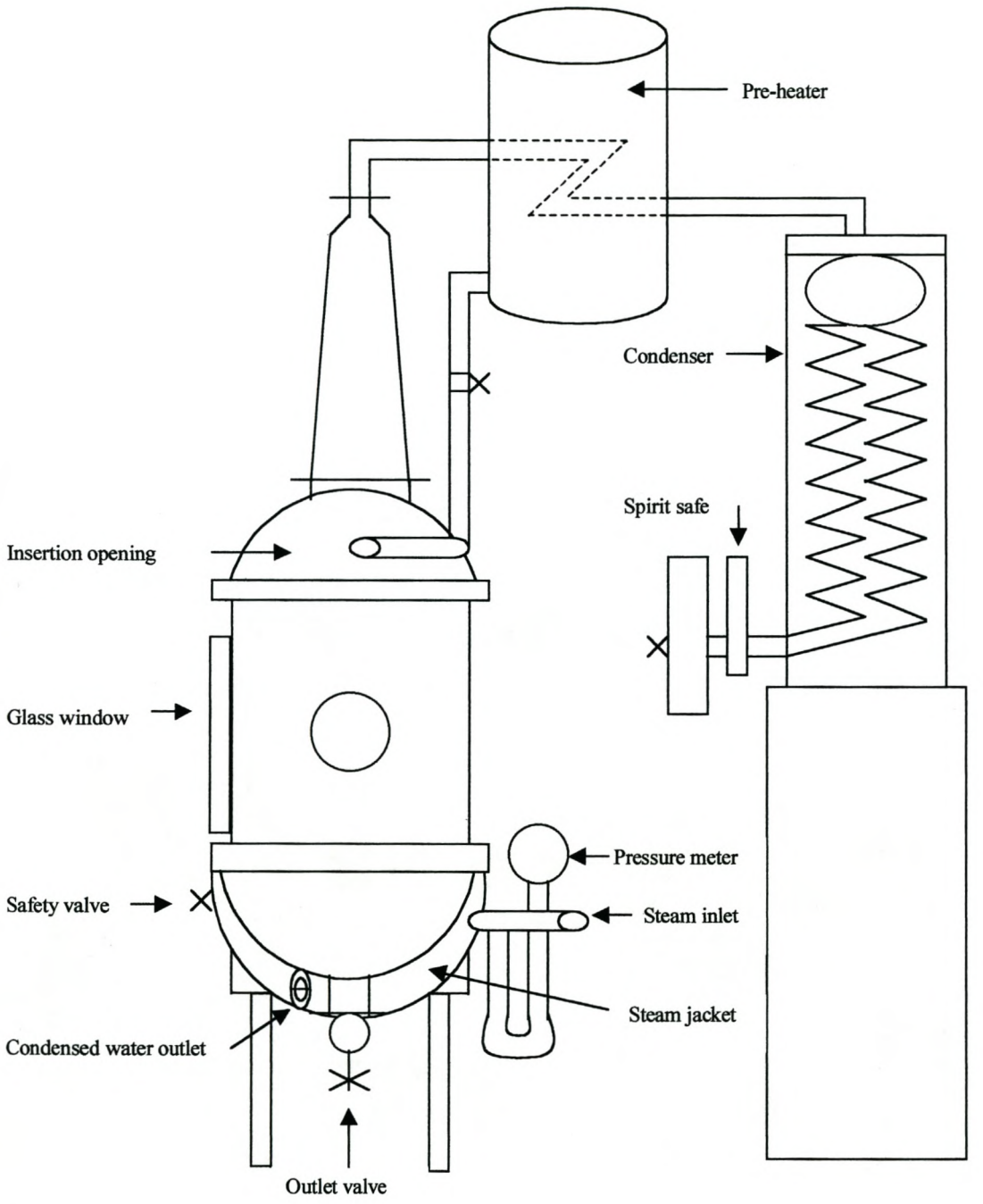


Figure 3. Schematic representation of a pot-still (Weitz, 2001).

and unrefined. It can, however, at least resist microbial spoilage until the next distillation. Ethanol and the secondary fermentation products are concentrated during this process and separated from the non-volatiles and most of the water. These secondary fermentation products include acetaldehyde, ethyl acetate and fusel oils (Bertrand, 1983; Weitz, 2001).

The second distillation is a slow process (10 to 12 hours) and the product is refined by distilling the crude brandy into three fractions (Anonymous, 1988; Weitz, 2001). These fractions are called the first flow, heart and faints, respectively (Figure 4). The first flow is collected within the first twenty minutes of the process and constitutes only about one percent of the original volume in the kettle (Weitz, 2001). During this period the coolers of the condenser are washed to rinse remaining fatty acids and residue from the pipes. The first flow contains lots of aldehydes and precious esters (Anonymous, 1988; Weitz, 2001). The heart is distilled until the alcohol percentage of the distillate reaches 70 to 75% alc/vol. The heart constitutes about one third of the original potkettle volume (Weitz, 2001). The faints, constituting about one third the volume of the original distillate, contain large amounts of fatty acids. The faints and the first flow will be redirected to a new feed of first distillate and undergo another second distillation (Anonymous, 1988; Weitz, 2001).

Aroma and flavour compounds develop during the different production stages (i.e. grape fermentation and distillation) of a distilled product, but the maturation process effectively determines the sensory properties of the matured, distilled beverages (Lehtonen & Jounela-Eriksson, 1983). The freshly distilled brandy is stored directly after distillation in oak-wood barrels. This helps to develop a smooth product, pleasant to the palate and rich in bouquet and flavours (Peuch & Moutounet, 1992). The process of maturation and aging is characterised by changes in colour and flavour of the maturing spirit and a decline of up to 5% per annum in the volume and alcohol content due to evaporation (Mosedale & Puech, 1998). Satisfactory maturation is achieved by extending the maturation period according to the characteristics of the raw distillate, size, wood origin, pretreatment of the cask and the environment (humidity and temperature) in which the spirit is matured (Delgado *et al.*, 1990; Mosedale & Peuch, 1998). In South Africa, casks made from oak wood from the Limousin region in France are most frequently used. These originate mostly from *Quercus pedunculata* and *Quercus robur* species, known for their coarser grain and therefore more rapid tannin and colour release (Anonymous, 1988; Weitz, 2001).

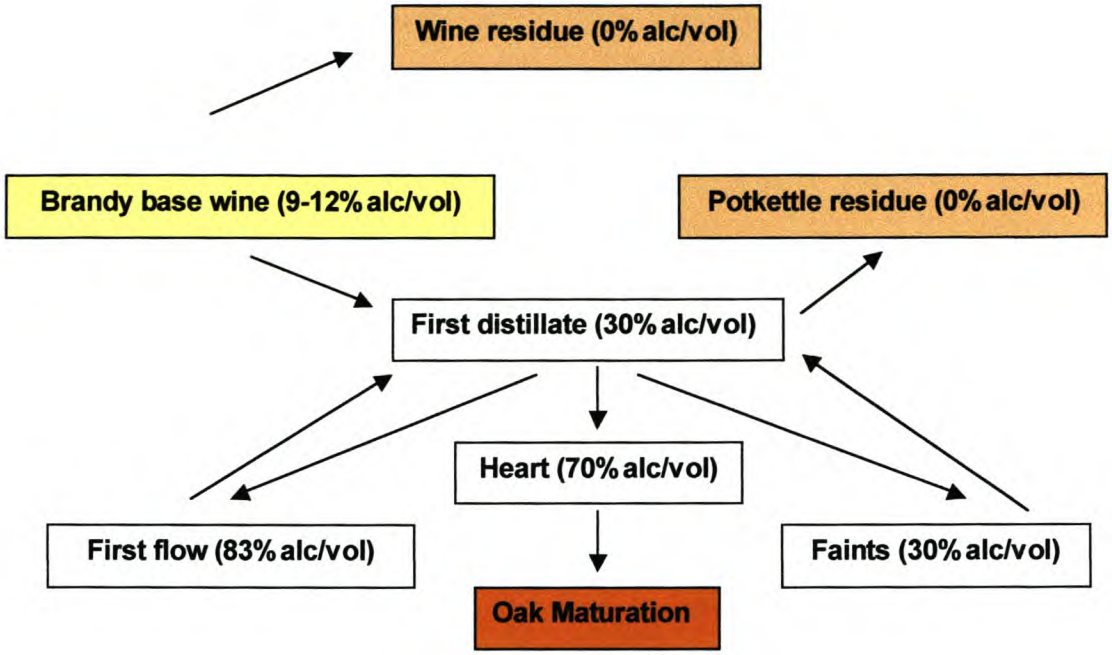


Figure 4. Block diagram of the brandy distillation process (Weitz, 2001).

Wood charring operations modify the macro molecular structure of wood, leading to degradation of polysaccharides and polyphenols (Guichard *et al.*, 1995; Cutzach *et al.*, 1997). It also introduces new classes of odoriferous volatile substances such as pyrazines, furans and phenols. Phenolic and furanic aldehydes are the most abundant of the numerous compounds identified in heated wood. Only vanillin (4-hydroxy-3-methoxybenzaldehyde), however, contributes actively to the aroma of the aged beverage (Cutzach *et al.*, 1997).

During the maturation process, a number of parallel processes occur that modify the composition of the original distilled spirit (Piggot *et al.*, 1992). Substantial quantities of phenolic and carbohydrate compounds are extracted from the wood and may undergo further alteration in the aqueous alcohol of the maturing beverage. Reactions occur between the original components of the distillate and the extracted wood material, thus illustrating the importance of both a suitable base wine as input product and controlled distillation and maturation processes. The extracted wood components are implicated in changes to sulphur compounds and the development of structure in the beverage (Piggot *et al.*, 1992; Mosedale & Peuch, 1998).

When the brandy is removed from the maturation stores after a minimum of three years, the distillate must be cold stabilised at –12°C for a few days to allow unstable solutes like calcium and iron compounds to settle. Thereafter these compounds are removed by filtration (Weitz, 2001).

Emerging from the maturation barrels is a considerable variety of amber-coloured beverages, each with a different character, flavour and depth of colour (Anonymous, 1988). To obtain a product and brand of established and recognised character, it is necessary to blend and correct the oak matured products. This is where the art of brandy making is essentially captured. The unique character of pot-still distillates originating from the wine grape cultivar, type and age of oak casks, lends itself to the development and blending of different styles of brandy. The brandy master classifies the brandy prior to blending based upon the sensory quality of the matured distillates, which can be described as a combination of the fullness, softness and taste, as well as flavour intensity of the products (Weitz, 2001). In South Africa, commercial lower cost brandies are bottled at 43% alc/vol of which at least 30% of the alcohol content must originate from three-year old pot-still brandy and the rest (not more than 70%) from neutral grape spirit (Anonymous, 1988; Weitz, 2001). Sweetening agents like sugar or honey (not more than 1.5% reducing sugars) can be added, as well as caramel to improve the colour. Bonifacteurs like fruit extracts (maximum 3% of alcohol content) can also be added (Weitz, 2001). Liqueur brandies are matured for 5, 10, 15 or more years and the oak-matured brandy content range between 35 to 95 percent of the final product (Anonymous, 1988). The age of the brandy indicated on the label must be equivalent to the period of wood maturation undergone by the pot-still distillate (Anonymous, 1988). These liqueur brandies are bottled at 38% alc/vol.

B2. Grape spirits

Distilling wine is a product made from grape juice during wine production utilising any wine grape variety (Steger, 2001). Juice retrieved from the first pressing of grapes during the pressing season is used for table wine fermentation. The juice from the second and third press will be used for distilling wine production. The distilling wine juice is fermented in a separate tank and juice obtained from poor quality grapes will also be added. Fermentation terminates when the residual sugar content is

exhausted, resulting in a product with an alcohol content ranging between 6 and 13% by volume (Steger, 2001; Weitz, 2001).

Wine cellars and cooperatives are granted yearly contracts to produce a specific volume of distilling wine for wholesale distilleries (Steger, 2001). Farmers are paid on delivery of the wine to the distilleries. Basic specifications are set to control the composition of the wine and incremental penalties are levied for wine that does not fall within these limits (Steger, 2001).

The raw wine is distilled in a column system (2-6 columns) in a continuous process that can run non-stop for up to ten months. With an inflow capacity of 4000 to 8000 litres per hour, 400 to 800 litres of spirits per hour are produced. The end product is a neutral alcoholic liquid without any flavour or taste except for the ethanol present. No aging or maturation is required and the product can be used immediately in the blending of brandies and manufacturing of other alcoholic beverages (Weitz, 2001).

C. An overview of the important quality parameters in distilling and brandy base wine

The Association of Official Analytical Communities (AOAC) and the Organization Internationale du Vin (OIV) define analytical reference methods that are often used within the wine industry (Zoecklein *et al.*, 1994). An issue of concern in the wine industry is increasing demands for regulatory compliance, thus increasing the demand for analyses of spoilage indicators, trace metals and agricultural chemical residues. The parameters regulated in distilling and brandy base wine are important for a variety of reasons and deserve further discussion.

C1. Alcohol

Ethanol as the most important alcohol, determines the body of any wine and affects its flavour (Burns, 1994). It serves as the basis of payment for distilling and brandy base wine and is defined by legal limits in various types of alcoholic beverages. The alcohol content of alcoholic beverages must be displayed on the labels of all products (Anonymous, 1996).

Hydrometry is the most common method used to determine the alcohol content of alcoholic beverages (Zoecklein *et al.*, 1994; Weeks, 1995). Two common interferences, sulphur dioxide and acetic acid may, however, cause problems during

analyses. Distillation combined with pycnometry, refractrometry and dichromate oxidation is also used relatively often. Enzymatic analysis involves the oxidation of ethanol in the presence of the enzyme alcohol dehydrogenase and nicotinamide adenine dinucleotide (NAD) yielding the reduced form of the coenzyme, NADH + H^+. This is a stoichiometric reaction under proper experimental conditions and the NADH produced can be determined spectrometrically at 334 nm. Densimetric procedures involve the determination of the specific gravity differences between the original wine sample and the alcohol distillate removed from the wine (Zoecklein *et al.,* 1994). Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC) (Zoecklein *et al.*, 1994; Weeks, 1995) and spectrophotometric flow injection systems (Mattos *et al.,* 1998; Rangel & Toth, 1999), have also been developed to determine the alcohol content in wines.

C2. Total acids

The acid content of wine is of importance regarding its flavour and its indirect influence on pH, colour, stability and shelf life of the product (Zoecklein *et al.*, 1994). A number of organic acids are present in wine and of these tartaric, malic, citric, acetic, succinic and lactic acids are quite common. These organic acids, along with others (propionic, butyric, gluconic) are commonly grouped into two categories, termed "fixed acidity" and "volatile acidity", respectively. Their sum is called "total acidity". The organic acid content of wine is traceable to three sources. The grape itself contributes tartaric, malic and to a lesser extent citric acid. Alcoholic fermentation of grape juice results in the formation of lactic, acetic, and succinic acids in addition to very small quantities of other Tricarboxylic Acid Cycle acids (Zoecklein *et al.*, 1994). Bacterial involvement produces significant amounts of lactic and acetic and under certain metabolic conditions propionic and butyric acids. Mould growth on the grapes may result in small amounts of gluconic acid in the finished product.

The AOAC titrametric procedure for total acids (sometimes referred to as "titratable" acids), involves a standard acid-base titration with standardized sodium hydroxide and phenolphthalein as indicator. More specific GC, HPLC, and enzymatic (Zoecklein *et al.*, 1994; Alonso *et al.*, 1998), and spectrophotometric (Mataix & Luque de Castro, 1999) methods also exist for organic acid determination in wines.

C3. Volatile acids

Acetic, propionic, butyric and sulphurous acids comprise the volatile acids in wine (Zoecklein *et al.*, 1994). Of these, acetic acid is the most significant and acts as an indicator of microbial spoilage and also plays an important organoleptic role (Burns, 1994; Zoecklein *et al.*, 1994; Anonymous, 1996). The principle source of acetic acid in stored wine is attributed to the growth of acetic acid bacteria from the family *Acetobacteraceae* (Zoecklein *et al.*, 1994; Ribéreau-Gayon *et al.*, 2000a). Heterolactic lactic acid bacteria may also produce significant amounts of acetic acid in addition to lactic acid and CO_2 when growing on glucose. Certain species of yeast, including *Saccharomyces* sp., *Brettanomyces* and its ascospore-forming counterpart *Dekkera,* also produce relatively large amounts of acetic acid under certain metabolic conditions (Zoecklein *et al.*, 1994; Ribéreau-Gayon *et al.*, 2000a). Post-fermentation sources of volatile acids are closely linked to oxygen availability and the development of headspace in the wine, providing both oxidative conditions and surface area which may support rapid growth of both bacteria and yeasts (Zoecklein *et al.*, 1994).

Steam distillation is frequently used to collect volatile acids during analyses. Thereafter, the distillate is titrated with standardized sodium hydroxide and the results reported as acetic acid (Zoecklein *et al.*, 1994). GC and HPLC (Zoecklein *et al.*, 1994; Alonso *et al.*, 1998), spectrophotometric (Mataix & Luque de Castro, 1999) and enzymic methods (Alonso *et al.*, 1998) are also commonly employed in research laboratories to determine the levels of the volatile organic acids in wine.

C4. Total sulphur dioxide

Sulphur dioxide is widely used in the wine industry as a chemical antioxidant and inhibitor of microbial activity (Zoecklein *et al.*, 1994). Sulphur dioxide is added to wine in liquid form SO_2 or potassium meta bisulphite. It exists in different states, i.e. free dissolved (SO_2), undissociated sulphurous acid (H_2SO_3), bisulphite (HSO_3^-) and sulfite (SO_3^-), which co-exist in chemical equilibrium (Anonymous, 1996; Ribéreau-Gayon *et al.*, 2000a). Only the free dissolved and the undissociated forms inhibit the growth or kill spoilage bacteria, fungi and wild yeasts. Free SO_2 also inhibits several enzymes responsible for the oxidation of polyphenols. The proportion of free SO_2 increases with decreasing pH (Anonymous, 1996; Ribéreau-Gayon *et al.*, 2000a). Certain yeasts produce small quantities of SO_2 during fermentation and even though

the amounts formed rarely exceed 10 $mg.L^{-1}$ to 30 $mg.L^{-1}$ can be produced in certain cases (Ribéreau-Gayon *et al.*, 2000a).

Several compounds found in juice and wine actively bind with sulphur dioxide, resulting in unwanted volatile substances. Acetaldehyde binds with free SO_2 (especially the bisulphite ion) to form a complex compound (bound SO_3), which has only weak anti-microbial properties (Liu & Pilone, 2000). This is a great problem in the distillation industry as this complex can accumulate in the fractions during distillation, imparting off-flavours into the product (Zoecklein *et al.,* 1994; Anonymous, 1996; Liu & Pilone, 2000; Steger, 2001).

The growing wine industry trend is towards the reduction of sulphur dioxide based on public health concerns, better quality fruit and the desire for malolactic fermentation in red wines. Although sulfites were historically generally regarded as safe, the United States Food and Drug Administration (FDA) has determined that the presence of sulfites in food and beverages poses a potential health hazard to a certain class of asthmatic individuals (Zoecklein *et al.*, 1994; Ribéreau-Gayon *et al.*, 2000a). As a result, the presence of sulfites in alcoholic beverages at levels exceeding 10 $mg.L^{-1}$ (parts per million) sulphur dioxide as determined by any method sanctioned by the AOAC, must be declared on the label. The control of sulphur dioxide in wines is also important, as excess can impart an undesirable taste on the nose and palate. In South Africa the levels are regulated through the Liquor Products Act, Act 60 of 1989, and the levels may not exceed the maximum permitted limits of 200 $mg.L^{-1}$ (Anonymous, 1996; Steger, 2001).

The internationally recognised AOAC method for total SO_2 in wine is the modified Monier-Williams aeration method (AOAC, 2000). This method involves the distillation of SO_2 (with nitrogen as a sweeping gas or with air aspiration) from the sample into peroxide and the subsequent titration of the formed H_2SO_4. The Ripper method for sulphur dioxide, which is more than 100 years old, employs standard iodine to titrate the total SO_2 in a sample (Zoecklein *et al.*, 1994). The method is extremely simple to perform, but universally recognised to be rather inaccurate. It is, however, still the most common method employed in winery laboratories to rapidly calculate sulphur dioxide levels (Zoecklein *et al.,* 1994). Flow injection systems with spectrophotometric detection (Cardwell *et al.,* 1992; Segundo *et al.,* 2000; Segundo & Rangel, 2001), a Voltammetric method (Cardwell *et al.*, 1992) and biosensor

systems (Nakamura *et al.*, 1993) have also been developed for total and free sulphur dioxide determinations in wine.

C5. Total phenols

Phenols are a large and complex group of compounds that are generally regarded as the main contributors to the colour and olfactory profile of wines (Zoecklein *et al.*, 1994; Ribéreau-Gayon *et al.*, 2000b). They are responsible for astringency and bitterness and serve as important oxygen reservoirs. According to Ribéreau-Gayon *et al.* (2000b), wine obtained from free-run juice as in the case with brandy base wine, essentially contains hydroxycinnamic acids as their phenolic components. These are the only phenolic components present in the pulp and the only phenolics to be readily released into the juice. The most significant phenolic compounds found in brandy are generated through the oak maturation process. As a result, lower levels of total phenols are preferred in the base wine (Steger, 2001).

The spectrometric measurement using Folin-Ciocalteu reagent is a common, non-specific measurement of the total phenol levels in wine. The reduction of the phenolic substances by the Folin-Ciocalteu reagent involves a reaction with a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$) and the subsequent spectrophotometric comparison at 765 nm. The procedure uses gallic acid as a standard reference compound and the results are correspondingly expressed as gallic acid equivalents (GAE) (Singleton & Rossi, 1965; Zoecklein *et al.*, 1994).

The first widely applied oxidation method for phenol determination in wine, involved a titration with potassium permanganate to an indigo-carmine end point (Zoecklein *et al.*, 1994). The permanganate index test serves as a crude evaluation of the phenolic content of wines. Another phenol measurement involves a simple screening test using Fe(II) Ammonium Sulphate (Zoecklein *et al.*, 1994). HPLC (Zoecklein *et al.*, 1994; Brenna *et al.*, 1998; Alonso *et al.*, 1998; Goldberg & Soleas, 1999; Mataix & Luque de Castro, 1999; Rechner *et al.*, 1999; Waterhouse *et al.*, 1999) and chromatography combined with mass spectrometry (Soleas & Goldberg, 1999) have found considerable application in the analyses of specific wine polyphenols.

C6. Reducing sugars

Reducing sugars can be described as those sugars containing functional groups capable of being oxidised and in turn, bringing about the reduction of other components (Zoecklein *et al.*, 1994). A reducing sugar, therefore, contains a free aldehyde or *alpha*-hydroxy ketone with reducing properties. Sugars in the free *aldo*- or *keto*-form or those that exist in equilibrium with these forms, fit into this category. The six-carbon sugars, glucose and fructose, are utilised by yeasts in alcoholic fermentation and are classified as reducing sugars. Certain pentoses, even though they are not fermentable by wine yeasts, contain oxidisable free aldehyde or ketone groups and are also regarded as reducing sugars.

Reducing sugar analyses play multiple roles in wine processing. The quality of the fermentable sugar remaining in the wine after completion of fermentation may be important for microbial stability (Zoecklein *et al.*, 1994). Certain spoilage yeasts like *Brettanomyces/Dekkera* and lactic acid bacteria grow at sugar levels below 2 $g.L^{-1}$. The monitoring of fermentable sugars in distilling material (i.e. distilling wine and brandy base wine) is of concern in the overall efficiency of the production plant (Zoecklein *et al.*, 1994).

Reducing sugars may be determined by a chemical method known as the Lane-Eynon procedure, that involves a reaction of reducing sugars with copper (II) in alkaline solution (Zoecklein *et al.*, 1994). Reducing sugars, such as glucose and fructose, reduce copper (II) to copper (I) oxide under alkaline conditions, with a subsequent colour change from yellow to red. Enzymatic and HPLC techniques have also been developed for more accurate determination of reducing sugars (Zoecklein *et al.*, 1994).

C7. Acetaldehyde

Acetaldehyde is the principle aldehyde present in wine (Zoecklein *et al.*, 1994). An intermediate in the microbial formation of acetic acid (i.e. the oxidation of ethanol during wine storage), acetaldehyde can disrupt the vapour/liquid equilibrium at the start of distillation (Zoecklein *et al.*, 1994; Steger, 2001). Under low-oxygen conditions and/or alcohol levels greater than 10% v/v, acetaldehyde tends to accumulate instead of being oxidised to acetic acid. The sensory threshold in wines ranges from 100 to 125 $mg.L^{-1}$. Above this threshold, acetaldehyde induces a strong, pungent, irritating odour in the wine (Zoecklein *et al.*, 1994; Liu & Pilone, 2000). This

necessitates the employment of a first (heads) fraction cut-off during brandy distillation to remove all the undesirable volatile compounds. Abnormally large amounts of acetaldehyde can, however, still accumulate into the heart fraction, producing unacceptable odours in the distilled product. Acetaldehyde actively binds with free SO_2 to form a complex compound (bound SO_3), as described in section C4 (p. 21) concerning total sulphur dioxide (Liu & Pilone, 2000). High levels of SO_2 before and during fermentation in juice and wine, result in higher levels of fixed acetaldehyde.

Acetaldehyde can be quantified by taking advantage of its strong binding affinity for sulphur dioxide in a modified titrametric method first proposed by Jaulmes & Espezel in 1935 (as cited by Zoecklein *et al.*, 1994). Acetaldehyde is easily separated from other constituents of wine on almost any semi-polar or polar column (Ferreira *et al.*, 1993). HPLC, colorimetric and enzymatic procedures have also been developed to determine acetaldehyde levels in wine (Zoecklein *et al.*, 1994).

D. Near infrared spectroscopy

D1. Introduction

Ian Murray (1999) stated the following: "*Food analysis was a relic of a bygone era using fragmentary, brutal chemical methods that reported too late to be any good in decision-making. If any topic was ripe for change, it was food, feed and forage testing. Either it would be done by NIR or it just wouldn't be done at all...Our enduring interest in NIR stems from the intellectual surprises it raises*". Today, NIRS with its unrivalled combination of speed, accuracy and simplicity, has found its own niche in the routine laboratories of food and beverage manufacturers worldwide. Advances in the enabling technologies of optics, electronics, computer hardware and software and especially chemometrics, have paved the way for more powerful NIRS analysers, resulting in more powerful spectral analyses (Workman, 1992; Osborne *et al.*, 1993).

The basic principles of spectroscopy and, therefore, NIRS involve the production, measurement and interpretation of spectra arising from the interaction of electromagnetic radiation with matter (Penner, 1994). Near infrared spectra of food constituents show broad bands, which comprise envelopes of overlapping absorptions corresponding chiefly to overtones and combinations involving C-O, O-H or N-H chemical bonds (Osborne *et al.*, 1993). A listing of the components in

foodstuffs reveals a majority of organic molecules that absorb light in the near infrared spectral region (750 – 2500 nm) (Norris, 1989; Ciurczak, 1992; Downey, 1998). A schematical representation of the electromagnetic spectrum, indicating the near infrared region, is shown in Figure 5.

A chemical mixture such as a foodstuff illuminated by electromagnetic radiation, will absorb radiation at certain frequencies through some of the atoms present in the mixture (Downey, 1995). By detecting this absorption, it is possible to describe the chemical composition of an unknown mixture without knowing the particular atomic structure that is responsible for the vibrational energy transmission. As this will occur at several frequencies for different chemical structures, it is possible to produce an absorption spectrum for a mixture that will represent the cumulative absorptions by all the constituents in that particular wavelength range (Ciurczak, 1992; Osborne *et al.*, 1993; Wetzel, 1998).

D2. The origin of near infrared absorption

Electromagnetic radiation can be described by a wave model, which states that a wave is a disturbance that transmits energy through a medium as shown in Figure 6 (Osborne *et al.*, 1993). The radiation in the form of electric and magnetic fields interacts with matter to give rise to a spectrum. Spectroscopic measurements involve the association between the individual frequencies of radiant energy and molecular motions, respectively (Osborne *et al.*, 1993). It is important to remember that the sensitivity of an optical absorption spectroscopic analytical method is dependent on the probability of a transition from the ground state to an elevated energy state within the molecule (Wetzel, 1998).

The quantum theory of Max Planck proposed in 1900, explains the energetic transition between two discrete energy states in terms of emission or absorption of discrete packets of energy, referred to as quanta (Ciurczak, 1992; Osborne *et al.*, 1993; Petrucci & Harwood, 1993). This energy can take on the form of electromagnetic radiation and the frequency of that radiation is related to the energy change (ΔE) depicted in equation 1.

$$\Delta E = E_1 - E_2 = h\nu \qquad \text{.....1}$$

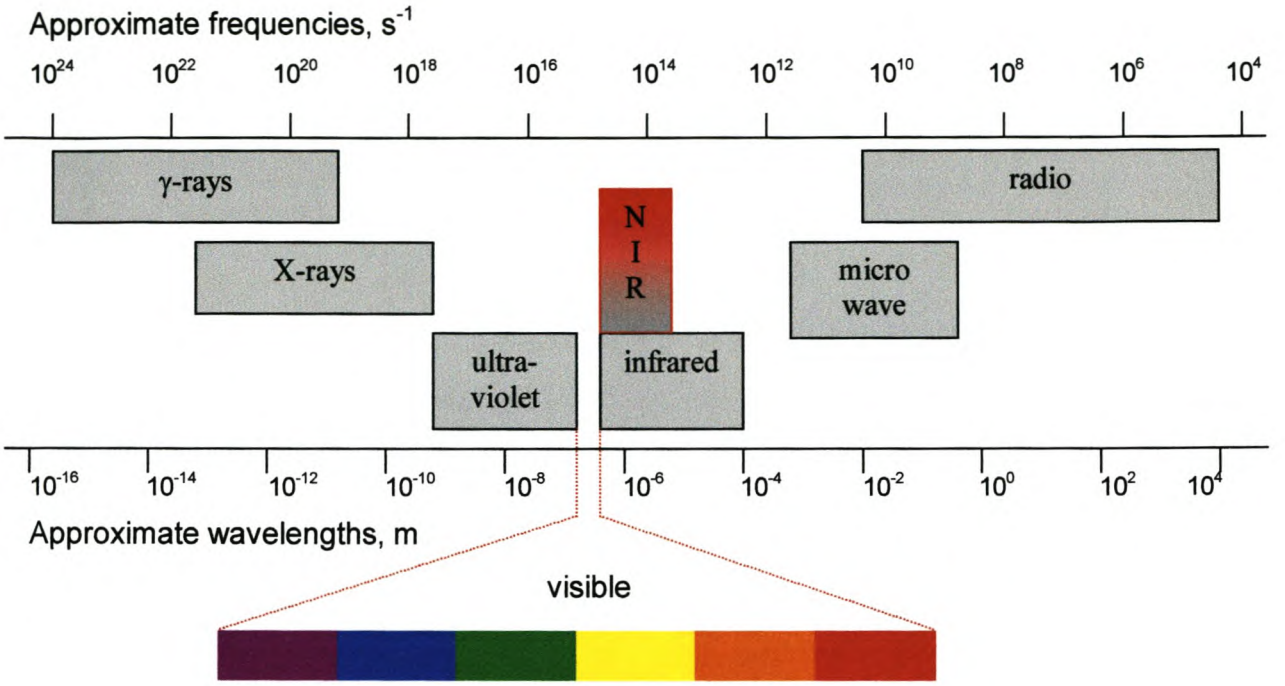


Figure 5. The electromagnetic spectrum indicating the near infrared region (adapted from Burns & Margoshes, 1992; Petrucci & Harwood, 1993; Penner, 1994).

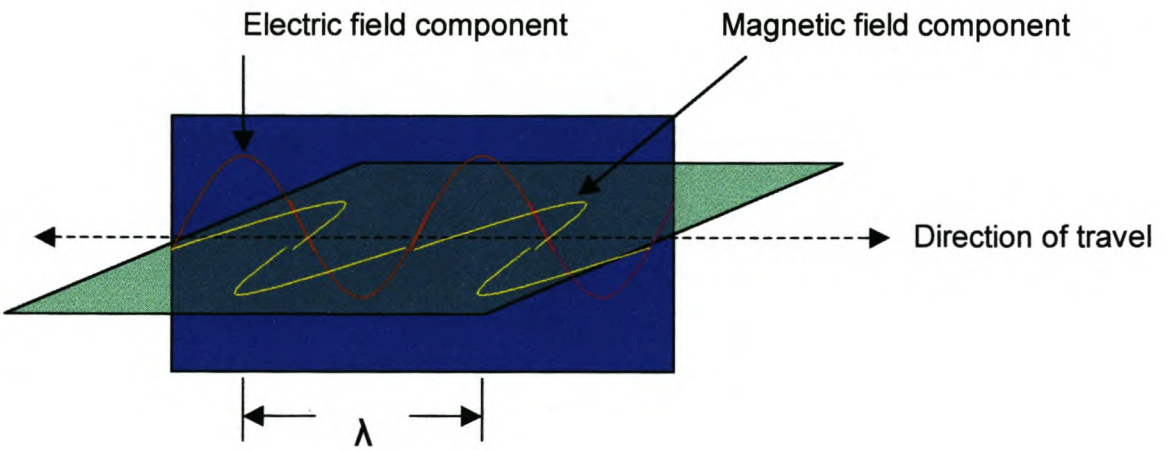


Figure 6. Schematic diagram of electromagnetic waves (Petrucci & Harwood, 1993).

where h = Planck's constant

v = Velocity

E_1 = Ground energy state

E_2 = Elevated energy state

A molecule in a low energy (E_1) or ground state, will undergo a transition to an elevated energy state (E_2) should a beam of radiation of a frequency which contains the specific energy increase needed, be directed at it (Osborne *et al.*, 1993). The energy difference between the two energy states is therefore accounted for by the absorption of some energy (ΔE) from the source beam at this specific frequency. An absorption spectrum can thus be produced by collecting the radiation after its interaction with the molecules with the aid of a detector (Osborne *et al.*, 1993). Spectral bands can be detected if the vibrations interact with the electromagnetic radiation, inducing temporary dipoles in polar bonds (Ciurczak, 1992; Osborne *et al.*, 1993).

D3. Molecular vibrations

Molecular energies take on different forms, but for the sake of infrared radiation, only vibrational motions need to be considered (Dillard & Goldberg, 1978). Stretching and contracting of bonds involve vibrations in which there is a continuous change in the interatomic distance along the axis of the bond between the two atoms (Dillard & Goldberg, 1978; Osborne *et al.,* 1993). Where a triatomic group of atoms RH_2 exists, stretching may occur symmetrically whereby the two R-H bonds vibrate in and out simultaneously or, asymmetrically when they vibrate in opposite directions. Vibrations involving a change in bond angle are known as bending and can further be classified into four types, namely scissoring, rocking (one part of the molecule vibrates with respect to the other), wagging and twisting, otherwise known respectively as symmetrical in-plane deformation, asymmetrical in-plane deformation, symmetrical out-of-plane deformation and asymmetrical out-of-plane deformation (Osborne *et al.,* 1993).

All of the vibrations of a molecule can be described as one or a combination of a certain number of fundamental modes of vibration (Dillard & Goldberg, 1978). When a molecule absorbs a photon of electromagnetic radiation, a vibration corresponding to one of its fundamental modes, becomes excited. Fundamental

frequency absorption involves the elevation from the ground state to energy levels where only one vibrational quantum number is 1 and the rest are zero (Ciurczak, 1992; Osborne *et al.*, 1993; Wetzel, 1998). Combinations may be considered as the average frequency of two adjacent molecular motions in a molecule. Bands due to fundamental vibrations are usually more intense than those due to combinations of vibrations (Dillard & Goldberg, 1978). Overtones normally originate outside the near infrared region and arise from the R-H stretching modes (O-H, C-H, S-H, N-H), instead of bending motions due to energy considerations (Ciurczak, 1992; Shenk *et al.*, 1992; Osborne *et al.*, 1993; Wetzel, 1998). Absorption bands in the near infrared region are the result of overtones and combination bands of fundamental vibrations in the mid-infrared (4000 – 600 cm^{-1}) region of the spectrum (Ciurczak, 1992; Shenk *et al.*, 1992; Osborne *et al.*, 1993; Wetzel, 1998).

D4. Chemical assignment of near infrared bands

Molecular vibrations appear as weak bands due to anharmonicity in the near infrared region (Ciurczak, 1992; Osborne *et al.,* 1993; Wehling, 1994; Wetzel, 1998). Wetzel (1998) states, *"In the near infrared we predominantly see the results of vibrations of light atoms that have strong molecular bonds"*. Chemical bonds involving the lightest atom, hydrogen, will vibrate with large amplitude when undergoing stretching, resulting in a motion that deviates considerably from harmonic oscillations (Ciurczak, 1992; Shenk *et al.*, 1992; Osborne *et al.*, 1993; Wetzel, 1998). This implies that the chemical structures observed in the near infrared region arise from overtones or hydrogenic stretching vibrations involving RH_y functional groups (eg. OH, NH or CH), or combinations involving stretching and bending modes of vibration of such groups. Consequently, near infrared absorption bands are limited mostly to organic molecules consisting of atoms such as nitrogen, oxygen or carbon attached to hydrogen.

Shifts in the exact location of absorption bands can take place due to changes in the chemical environment (Wetzel, 1998). Certain constituents, like salt for example, may perturb the bonding scheme of a molecule that has near infrared absorption (eg. water), resulting in drastic frequency shifts and amplitude changes in the overtone bands i.e. in the near infrared region (Wetzel, 1999; Murray, 1999). In food and beverage analyses, near infrared spectroscopy measures absorbencies in a complex matrix. Data is acquired for the whole matrix and not just the analyte or

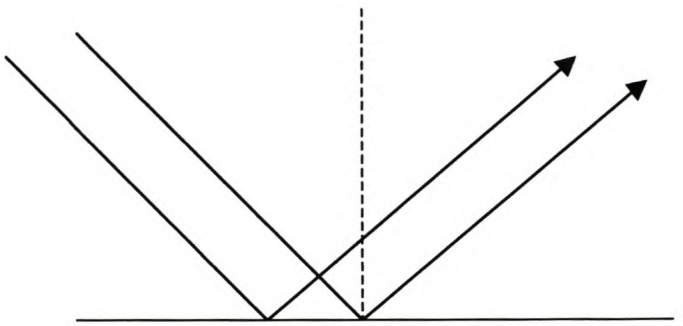
constituent in question, therefore, it may be possible to detect and quantify compounds that do not have near infrared absorption bands to begin with (Wetzel, 1998; Murray, 1999). Due to this type of environmental effect, compounds that do not have near infrared absorption bands may be detected in this spectral region if they produce band shifts in absorbing molecules in the sample matrix.

Numerous regions occur in the near infrared where wavelength reproducibility is high, signals maximised and noise minimised, all due to the weak bands in the NIR region (Wehling, 1994; Wetzel, 1998). This implies that the optical responses are sensitive to the environment of the absorbing molecules and the number of those molecules present. Quantitative measurements can therefore be made and successfully correlated to chemical data obtained through conventional analytical techniques (Osborne *et al.*, 1993; Wetzel, 1998).

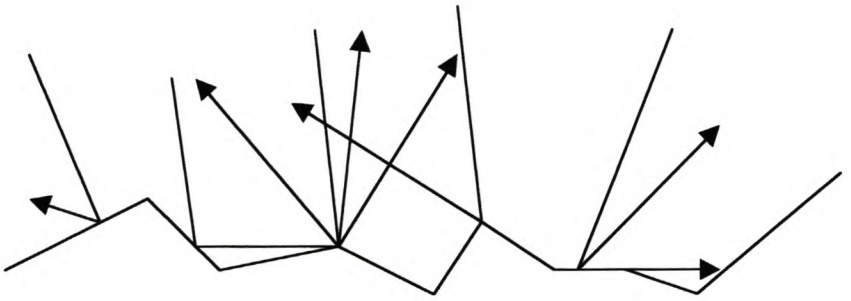
D5. Radiation can be absorbed, transmitted or reflected

Reflectance

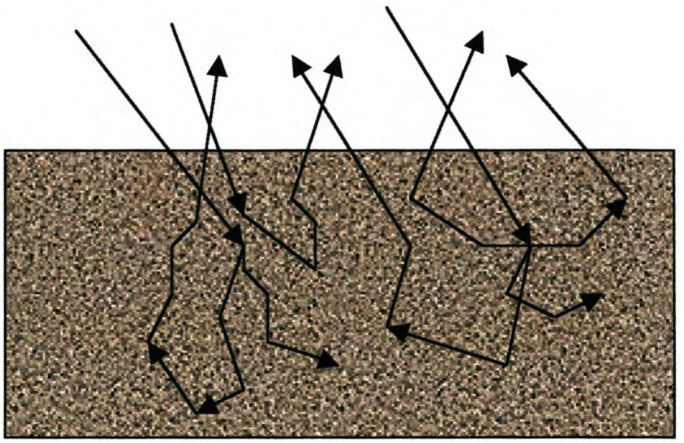
Non-absorbing and opaque samples (smooth surfaces) will reflect incident radiation in a manner similar to reflection of a light beam by a mirror as seen in Figure 7a (Osborne *et al.*, 1993). The state of the incident beam, the reflected beam and the normal to the mirror at the point of incidence, all lie in the same plane. The angle of incidence is equal to the angle of reflection. In the case of a sample with a matt surface, the boundary between the sample and the surrounding medium may be considered to consist of a series of small interfaces oriented at all possible angles to the normal (Figure 7b). If radiation transmitted through the first interface into the sample undergoes absorption, the transmitted radiation will be attenuated according to the Beer-Lambert law (Osborne *et al.*, 1993; Penner, 1994). The emerging radiation from the interface becomes diffused by random reflections, refractions and scatter at further interfaces inside the sample (Figure 7c). Scattering can occur when discrete particles are encountered within the sample. Destructive interference becomes incomplete and the radiation is propagated in all directions (Osborne *et al.*, 1993). Reflectance measurements penetrate only 1-4 mm of the front surface of a sample (Workman & Burns, 1992).



a) Smooth surface



b) Matt surface



c) Diffuse (body) reflectance

Figure 7. The effect of sample surface on reflection of radiation (Osborne *et al.*, 1993).

Transmittance

Transmission involves the passage of light through a sample, mostly transparent liquids, powders and slurries (Wetzel, 1998). The variation in intensity of transmission with wavelength gives rise to a transmittance spectrum. The fraction of radiation (P/P_O) transmitted by a sample is measured and referred to as the transmittance (T). The spectrum is the difference between the raw transmittance measurement of the sample and the raw transmittance measurement of the reference material (Workman & Burns, 1992). In transmittance measurements, the entire path length of sample is integrated into the spectral measurement, thereby reducing errors due to non-homogeneity of samples. The transmittance can simply be converted to absorbance (A), which is defined by equation 2.

$$A = \log 1/T = \log (P_o/P) \qquad \text{.....2}$$

where P_o = total radiant power incident on the sample

P = radiant energy absorbed

The Beer-Lambert law in equation 3 describes the attenuation of the transmitted radiation by an absorbing sample. The law states that the fraction dP/P of radiant energy P absorbed by an infinitesimal thickness of sample, is proportional to the number of molecules dn in that thickness.

$$dP/P = k\, dn \qquad \text{.....3}$$

where dP/P = the fraction of radiant energy P

P = radiant energy absorbed

dn = number of molecules

Another form of Beer's law (equation 4) states the direct relationship between the absorbance and molecular concentration, if an accurately defined sample thickness is defined and absorptivity calculated through a series of samples of known concentration.

$A = abc$ 4

where A = absorbance (optical density)
a = absorptivity coefficient or molar absorptivity
b = sample path length
c = concentration of the molecules in sample

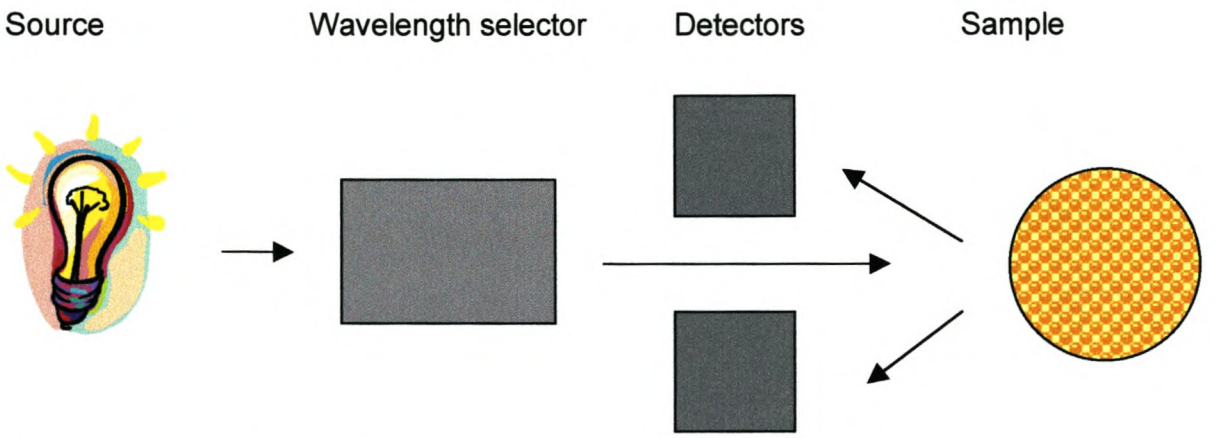
If the Beer-Lambert relationship holds true, a plot of absorbance against concentration will result in a straight line through the origin, with slope *ab*. From that, a (absorptivity) can easily be determined (Osborne *et al.*, 1993; Penner, 1994).

D6. Instrumentation

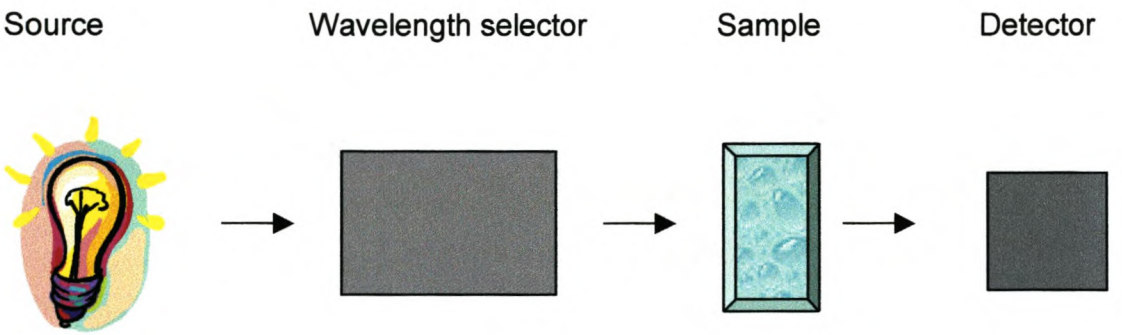
The method by which light is spectrally modulated or selected, defines the optical operating principle of a near infrared spectrophotometer (Osborne *et al.,* 1993). All NIRS instruments may be classified into one of the following groups; dispersive, interferometric and non-thermal (Workman & Burns, 1992; Osborne *et al.*, 1993; Wetzel, 1998). The first two groups generally employ broadband, thermal radiation produced by an incandescent filament. The third group consists of non-thermal sources where wavelength selection is inherent in the source's spectrally narrow emitting range (Osborne *et al.*, 1993). Measuring instruments with different arrangements for sample presentation, wavelength selection, signal detection, and readout exist. The basic configurations for reflectance and transmittance detection are shown in Figure 8. The following section deals with the different types of NIRS equipment and especially those that will be employed in the study, interferometers and diode arrays, representing interferometric and non-thermal instrumentation, respectively.

Filter instruments

The first commercial instruments introduced in the early years of NIRS were based on interference filters (Osborne *et al.*, 1993). The analysers were mostly equipped with quartz tungsten halogen sources and a lead sulphide detector that required a phase sensitive amplifier and had a digital readout (Workman & Burns, 1992; Wetzel, 1998). The interference filter is tilted away from the perpendicular to the incident



a) Near infrared reflectance



b) Near infrared transmittance

Figure 8. Basic instrument configurations for a) reflectance and b) transmittance (Workman & Burns, 1992; Osborne *et al.*, 1993).

source, allowing the scanning of a limited wavelength region (Norris, 1989). These filter instruments can utilise a selected narrow wavelength range, enabling the measurement of well-defined properties (like moisture and protein) in products like whole wheat and flour at their specific absorbing wavelengths (Norris, 1989).

Grating monochromators

Dedicated dispersive (grating-type) scanning NIRS instruments have been available for more than twenty years (Workman & Burns, 1992; Wetzel, 1998). A dispersive system is one where wavelengths of light are separated spatially, usually by using prism designs. The basic design consists of tungsten-halogen source lamps, single monochromator with a holographic diffraction grating and uncooled lead sulphide detectors (Norris, 1989; Workman & Burns, 1992; Osborne *et al.*, 1993; Wetzel, 1998). The light beam is chopped into an alternating on-off beam to produce angular dispersion. Synchronous detection is used with the photocell signal to reduce the effects of temperature change, amplifier drift and stray radiation (Norris, 1989; Wetzel, 1998). The large-area lead sulphide detectors are used to measure the radiation reflected from the sample. These signals (representing reflectance wavelengths) are received from the detectors and are amplified and converted to digital signals, which is then coupled into a data processor and stored (Norris, 1989).

Interferometers

The conditions for optical interference are created by an interferometer by splitting light into two beams and then recombining them after a path difference has been introduced (Osborne *et al.*, 1993). A beam of radiation from the source entering the interferometer encounters a beam splitter (Figure 9), which divides the light equally between the two beams and recombines them (Van de Voort, 1992; Osborne *et al.*,1993; Wetzel, 1998). In this optical device about half the incident radiation is transmitted and the other half reflected. A second moving mirror is encountered in one pathway and renders the magnitude of that pathway variable as a function of time. The difference in the two path lengths at different positions of the second mirror causes interference (Osborne *et al.*, 1993; Wetzel, 1998).

Near infrared absorption spectrophotometry requires a broad, constant source of energy, spanning the spectral region from the visible region (400-750 nm) to

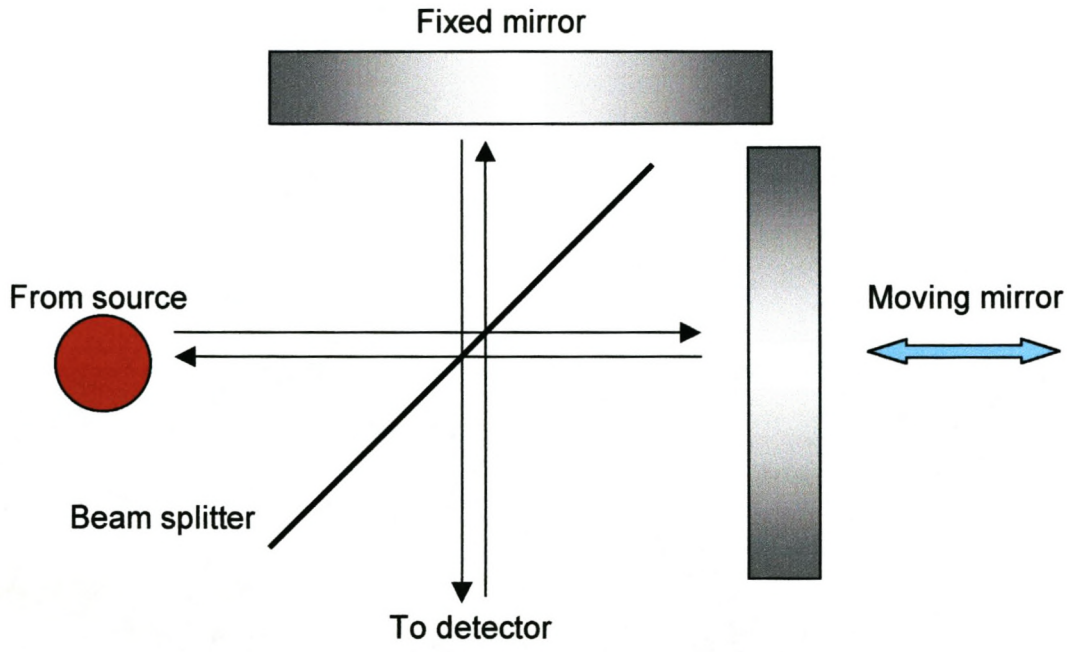


Figure 9. A schematic diagram of the Michelson interferometer (Van de Voort, 1992; Osborne *et al.*, 1993).

3000 nm (Osborne *et al.*, 1993). A quartz tungsten halogen lamp normally acts as the radiation source and an internal near infrared DTGS (deuterated triglycine sulphate) detector serves as standard for transmittance measurement. A lead sulphide photoconductive cell serves as a detector (Anonymous, 1997a; Wetzel, 1998). The signal resulting from the detector, known as the interferogram, contains all the information required to construct a spectrum through Fourier transformation. The advantages of Fourier transform near infrared spectroscopy instrumentation in comparison to traditional dispersive equipment like the grating monochromator, include wavelength resolution together with higher sensitivity and precision of both wavelength and intensity readings (Wetzel, 1998).

Diode arrays

Electronic wavelength switching as a means of scanning the spectrum, in contrast to the classical grating monochromator where physical rotation of the grating is necessary to aim a particular ray through the monochromator exit slit before traversing the sample and hitting the detector, enables random wavelength access which speeds up the scanning process dramatically (Wetzel, 1998). The sample is illuminated by chopped, high-intensity, broadband energy (white light) from a tungsten halogen lamp (Perten, 1999). The white light travels through the sample and proceeds via a sensor module through the entrance slit of a polychromator with a concave grating. After exiting the dispersive device, the resultant spectrum falls simultaneously upon each of the sensing elements of a detector array.

The rapid response photodiode detector consists of indium-gallium-arsenide (InGaAs) diodes, spaced in uniform wavelength intervals to form an array that covers the optical range from 950-1700 nm (Wetzel, 1998; Perten, 1999). Silicon diodes are very sensitive in the near infrared region and are included to cover the wavelength range 400-950 nm (Perten, 1999). The spectrum is captured by means of a "freeze-frame" motion through a separate analogue signal channel provided for each diode in the array. A reference channel for stray light and detector offset throughout the spectrum is provided for continuous correction of background effects by feeding a portion of source energy captured during a "dark" interval" of the sample through to the sensor module (Perten, 1999).

D7. Sample presentation of liquids

The measurement of liquids is usually less complicated than that of solids, even though wider absorption ranges may be encountered (Osborne *et al.*, 1993). Spectral data acquisition in liquids are performed through transmittance, where the incident light illuminates one side of the sample and the unabsorbed portion of the incident light is channelled to the detector on the opposite side (Kawano, 2002). In transmittance measurements, the entire path length of a sample is integrated into the spectral measurement (Workman & Burns, 1992). The path length of the cuvette of a flow-through cell can be adjusted to optimise the absorption sensitivity. It is important to take temperature changes into consideration, therefore, a controlled environment is desirable. The path length of the cell depends on the wavelength region and samples used (Kawano, 2002). A supply of desiccant placed within the system removes any water vapour that may interfere with the sample (Anonymous, 1997b).

Most instrument manufacturers produce a range of cells manufactured from glass or quartz. Quartz does not absorb in the near infrared region and thereby provides a smooth uniform surface from which reflection can occur (Wehling, 1994). The Beer-Lambert law is obeyed, unless a very turbid liquid or one containing lots of particles is encountered (Osborne *et al.*, 1993).

E. Chemometrics

E1. Introduction

Chemometrics is a branch of applied statistics, which specifically involves the mathematical treatment of chemical or spectroscopic data to extract information and uses statistical methods that arose from mainstream statistical theory (Downey, 1995). Workman (1992), states "*Chemometrics is a term applied to the generic discipline involving computers and mathematics to derive meaningful chemical information from samples of varying complexity.*"

Near infrared spectroscopic analysis is dependant on the development of an empirical linear relationship in which the concentration of a constituent is related to optical measurement, usually expressed in absorbance or, in the case of reflectance measurements, log 1/reflectance (Wetzel, 1998). The chemometric operations that are used to relate the optical measurements to quantitative data, aid the

development of a calibration model that can be used for future predictions of a specific constituent concentration.

The main goal of the calibration procedure is to find the best fitting equation for the samples in the calibration set (Martens & Næs, 1989; Westerhaus, 1989a). This is a multiterm linear expression with appropriate coefficients that makes appropriate analytical use of optical data. The first step in the calibration process is to construct a data matrix X from the instrument responses at selected wavelengths for a given calibration sample set. A matrix of concentration values, **Y**, is obtained through a reference method (Beebe & Kowalski, 1987; Geladi, 2002). Multivariate calibration involves a range of mathematical activities to find the relationship between one or more response variables *y (*or **y**) and a vector of predictor variables **x** as seen in equation 5 (Geladi, 2002).

$$y = g(\mathbf{x}) \quad \text{or} \quad \mathbf{y} = g(\mathbf{x}) \qquad \text{.....5}$$

Spectra in the near infrared region generally contain considerable amounts of information about the physical and chemical properties of molecules (Shenk *et al.*, 1992; Downey 1998, Katsumoto *et al.*, 2001). One of the biggest challenges faced when analysing spectral data is to eliminate or reduce the noise from the spectra. This not only eases visualisation of the information contained in the spectra, but also maximises the utilisation of the useful data (Wetzel, 1998; Katsumoto *et al.*, 2001).

Overlapping of the many bands in the near infrared region, evidenced as multicollinearity, complicated the analysis of the spectra in the past (Osborne *et al.*, 1993). Due to the advancement of chemometric tools and powerful computer processors in recent years, near infrared spectra can now be analysed with great speed and accuracy. The main task of the computer in NIRS, aside from driving the instrument or collecting data, is to interpret the spectra using a variety of multivariate mathematical techniques (Workman, 1992). Prediction models can then be constructed whereby the values of unknown samples can be predicted in future. The accuracy of the calibration model is highly dependant on the accuracy of the results obtained by the reference method (Martens & Næs, 1989; Workman, 1992; Osborne *et al.,* 1993; Wetzel, 1998). The task of calibration can be somewhat elementary when dealing with one- or two-component matrices, but it becomes quite complex when dealing with samples of biological origin (Workman, 1992). Minute changes in

absorbance may indicate large concentration changes and interfering bands overlap severely, causing interference/interaction with bands representing the constituents of interest. The successful deployment of NIRS involves the multidisciplinary approaches of the analytical chemist, statistician and computer programmer.

E2. Pretreatment

Proper pretreatments are usually imposed on experimental spectral data to reduce noise, correct baseline variations and enhance the resolution (Katsumoto *et al.*, 2001). Near infrared spectrophotometers have very high signal-to-noise specifications, resulting in spectra that contain very little random noise. Unwanted information due to scatter effects can, however, obscure useful information and need to be eliminated. Pretreatment methods should facilitate the improved spectral analysis of data, rather than reduce or eliminate useful information.

NIRS is concerned with observing differences between two or more samples of milliabsorbance units. The data obtained is, however, only meaningful if the noise level is kept to a few microabsorbance units (Wetzel, 1998). Noise arises from processes related to normal physical and chemical interferences and is usually associated with the instrument rather than the sample. Smoothing methods, such as the Savitzky-Golay method, can be employed to reduce the high-frequency noise. Wavelet transforms can be employed to remove high and low frequency noise, as well as localised noise due to phenomena like scattering (Katsumoto *et al.*, 2001).

Light scattering and density variations cause baseline fluctuations in transmittance spectra. Corrections for these baseline changes include derivative methods and multiplicative scatter correction (MSC). The most commonly used derivative method is the second derivative calculation, which enables the investigation of individual band shifts. Second derivative calculations are also powerful in removing additive and multiplicative baseline variations in the spectra. MSC, estimated by least squares regression, corrects spectra according to a simple linear univariate fit to a standard spectrum. Essentially MSC improves the linearity of the variables in NIRS (Wetzel, 1998; Fearn, 1999; Katsumoto *et al.*, 2001). Other baseline correction methods include de-trending, standard normal variate (SNV) and orthogonal signal correction (OSC), which enable the removal of interfering variations present in spectra (Wold *et al.*, 1998; Fearn, 1999; Katsumoto *et al.*, 2001). SNV, which is linearly related to MSC (Wold *et al.*, 1998), processes each spectrum by

subtracting the mean of the spectra from the spectral values obtained at each wavelength. The centered spectra are subsequently scaled by the standard deviation, *si,* of these spectra (Katsumoto *et al.*, 2001).

Resolution enhancement enables the separation of overlapping bands and elucidates the existence of obscured bands. Derivative methods, mean centering, and Fourier self-deconvolution are all used as resolution enhancement methods, even though these are not regarded as pretreatment methods (Katsumoto *et al.*, 2001). Normalisation transforms the spectral points on a unit hypersphere according to a constant Euclidean norm (equation 6), thereby presenting all the data in approximately the same scaling (Katsumoto *et al.*, 2001).

$$Xj, norm = xj / \|x\| \qquad \text{.....6}$$

where $\|x\|$ = Euclidean norm of the spectral vector x

E3. Multiple linear regression (MLR)

The function $g(\mathbf{x})$ in equation 5 is difficult to determine because of the large amount of variables in **x** and the amount of noise found in the data of y and **x** (Geladi, 2002). MLR is an approximation model that incorporates the linear polynomial equation 7.

$$y = b_0 + b_1 x_1 + b_2 x_2 + \ldots + b_k x_k + f \qquad \text{.....7}$$

where b_0 = an offset

$b_k (k=1,\ldots.K)$ = regression coefficients

f = a residual

The individual variables contribute independently to the information contained in y (Martens & Næs, 1989). The MLR method is the most popular method used in systems where there are no non-linearities, no interferences or interactions with other constituents and a low level of noise disruptions (Martin, 1992). A problem with MLR is the fact that outliers can easily be concealed during the fitting process by being drawn into line with the other points. This happens at the expense of distorting the equation (Osborne *et al.*, 1993).

E4. Principal component regression (PCR) and partial least squares regression (PLS)

PCR and PLS are methods based on latent variables, in other words, principal components are chosen to explain the variability in *y*, rather than a large number of **x** variables (Geladi, 2002). When expecting the x-variables to be collinear, the matrix X will have some dominating types of variability that carry most of the available information. Thus, only a few of the principal components, called regression factors, are used in the regression equation (Martens & Næs, 1989). The purpose of principal component analysis is to express the main information in the variables **x** by a lower number of variables, referred to as the principal components. As successive components are calculated, each component accounts for the maximum possible amount of residual variance in the dataset (Mark, 1992a). The calculation involves orthogonal (i.e. uncorrelated) axes. Weights are the constants that are used as multipliers for each of the original **x** datapoints to achieve their transformations in the new multi-dimensional space. These are referred to as eigenvectors. The first principal component often accounts for more than 90% of the variance (Wetzel, 1998). Each successive component accounts for less than the previous one. The American Society for Testing and Materials defines principal component analysis as a mathematical procedure, used to resolve sets of data into orthogonal components whose linear combinations approximate the original data to any desired degree of accuracy (Mark, 1992a).

One of the most important factors involving near infrared calibrations is to determine the number of principal components that will capture the essential information in the matrix X (Martens & Næs, 1989). Overfitting is the pitfall to avoid when performing principal component calculations (Osborne *et al.*, 1993). If too many components are used, too much of the redundancy of the spectrum is incorporated into the model, and the equations become too calibration-data dependent (Næs & Issakson, 1991a). Performance on the calibration set will continue to improve as long as factors are added, but models with many factors rarely predict well.

The difference between PCR and PLS involves the way in which the variables are decomposed into factors (Martin, 1992). Decomposition in PCR is performed independently of the constituent reference data, whereas PLS calculation involves the incorporation of the concentration data into the factoring (Martens & Næs, 1989).

According to Osborne *et al.* (1993), the PLS method uses a criterion during the construction of factors that balances the need to capture as much variation in the spectral data as possible and correlates the factors with the reference data. PLS therefore tends to produce solutions that require fewer factors than calibrations of comparable performance produced by PCR (Osborne *et al.*, 1993). PLS1 refers to regression for a single Y-variable, whereas PLS2 involves regression for several Y-variable (Martens & Næs, 1989).

The essence of PCR and PLS lies in their construction of factors from the original spectral data, keeping in mind the aim of reducing the spectral data without discarding useful information and avoiding overfitting (Osborne *et al.*, 1993).

E5. Qualitative Discriminant Analysis

Principal component scores for materials tend to cluster in multidimensional space, in a similar fashion to the way the data points representing individual wavelength measurements may cluster (Mark, 1992b; Downey, 1996). Principal component analysis models are constructed using the entire dataset obtained from all the measurements of the different samples to be distinguished (Mark, 1992b).

Spectroscopic methods with specific reference to NIRS, have demonstrated potential during discriminatory studies to determine the authenticity of several foodstuffs and food ingredients (Downey, 1996). NIRS has shown great potential in discriminating between frozen and frozen-then-thawed beef (Downey & Beauchêne, 1997a; Downey & Beauchêne, 1997b), Robusta and Arabica coffee (Downey *et al.*, 1994; Downey *et al.*, 1997) and virgin olive oils from Crete, the Peloponese and other Greek islands (Downey & Flynn, 2002) through factorial discriminant analysis. PCA of a series of nine different vegetable oils (Sato, 1994), seven different volatile oils (Kiskó & Seregély, 2002) and different waxes used in the dairy industry to coat cheeses (Barzaghi *et al.*, 2002), revealed clear clustering according to sample type. Successful discriminant analysis of commercial teas with and without an adulterant tea (Osborne & Fearn, 1988) and Brazilian, Israeli authentic and Israeli adulterated orange juices (Shilton *et al.*, 1998) were achieved with PCA. In the dairy industry, NIRS has been successfully applied to detect adulteration of milk with strange fats (Sato *et al.*, 1990) and substances like water, sodium chloride and skim milk powder (Pedretti *et al.*, 1993). Reasonable results were also obtained during studies to

discriminate between Basmati and other long-grain rice samples (Osborne *et al.*, 1997) and bread baking quality of different wheat varieties (Devaux *et al.*, 1987).

Soft independent modelling of class analogy (SIMCA)

Apart from problems relating to simple classification, SIMCA is applied to a more general class of discriminatory issues , e.g. identification. Data describing samples from each of the relevant number of classes or groups e.g. different meat species or coffee varieties, are collected and separate PCA models are calculated for each of the groups for which qualitative analysis is desired (Mark, 1992b). SIMCA first centers and then compresses raw data by means of PCA (Mark, 1992b; Downey & Beauchêne, 1997b). A multidimensional space is constructed containing the scores corresponding to each group. Mahalanobis distances based on the principal component scores are calculated for every sample to determine the distance from the cluster centroid in the dimensional space. Each cluster model treats new samples separately and an assessment of cluster membership is made on the basis of the distance to the cluster centroid. An F-test is employed to measure the degree of similarity of an unknown sample spectrum to sample spectra in each modelled cluster, allowing an estimate of confidence to be attached to any identification decision (Downey, 1996; Downey & Beauchêne, 1997b). The sum of squares of a residual spectrum can be compared to the variance within the class, providing a measure of certainty accompanying every identification (Downey, 1996; Downey & Beauchêne, 1997b). The spectrum residual, which is an indication of how much of the spectrum is not accounted for by the model, provides a reliable and sensitive measure of class membership. By combining two residual distances, the critical probability whether an unknown sample belongs to the specified class, is tested.

Reported applications of SIMCA to food classification using near infrared spectra are few in number due to lack of successful results (Downey, 1996). SIMCA has, however, been successfully applied for the diagnosis of mastitis in dairy cows on the near infrared spectra of milk, blood and urine samples (Tsenkova & Atanassova, 2002).

E6. Validation

It is important to assess the future performance of every calibration model. This is done by collecting a further set of samples (prediction or validation set) with known

reference values and using the calibration models to predict the constituent values of these samples (Osborne *et al.*, 1993). The comparison of the NIRS prediction values obtained for the constituents or properties with the measurements obtained for the reference method, is called validation of the calibration process or prediction testing (Næs & Isaksson, 1991b). The aim is to fit the NIRS and reference values onto a straight line and compare it statistically to a theoretically perfect line through the origin at 45° to the axes. External validation or prediction testing requires a separate large and representative set of test objects in order to give relevant and reliable estimates of the future prediction ability of the model (Martens & Næs, 1989; Westerhaus, 1989b). It is, however, not always possible, as multivariate calibration is often done because the traditional reference method for measuring *y* is too expensive, slow or otherwise undesirable. It would be most economical to use all available data for both calibration development and for prediction testing (Martens & Næs, 1989).

Internal validation concerns validation using the same sample set as that used for calibration development. An assessment based on internal validation is, therefore, not the same as prediction testing (Martens & Næs, 1989). The ideal would be to test the predictive ability on new objects, but cross validation and leverage correction give sensible results with high information about the prediction ability. The danger of underestimation is an ever-present problem as the same data is used for both model fitting and testing (Martens & Næs, 1989; Næs & Isaksson, 1991b). Cross validation is a very reliable validation method. It seeks to validate the calibration model on an independent test dataset, but contrary to external data, it does not waste data for testing only. For cross validation, successive samples are deleted from the calibration set. In partial-cross validation, samples are removed in groups, while in full-cross validation, all samples are removed one at a time. After every deletion, a calibration is performed on the rest of the samples, before being tested on the removed samples. The first sample(s) is (are) then replaced into the calibration data and the next sample(s) removed. The procedure continues until all the samples or sample groups have been deleted once (Martens & Næs, 1989; Næs & Isaksson, 1991b).

An important part of equation 7 is the parameter *f*, called the residual, and validating the model by its residual is part of the validation process (Geladi, 2002). By plotting the residuals against predicted **y**, outliers, heteroscedastic behaviour and

bias are detected and by plotting the residuals against time, instrument drift can be detected. Studying the residuals can also identify over- or underfitting. The residual standard deviation from the regression is the most obvious indication of the model's possible future performance (Osborne *et al.*, 1993).

The ultimate goal of a calibration model is to predict unknown values accurately and precisely (Geladi, 2002). The test set should give a realistic representation of samples that may be encountered in future (Osborne *et al.*, 1993). The test of a regression model is how well it predicts a well-chosen test set. A simple graph of predicted versus measured results may explain various situations that can arise (Geladi, 2002). In Figure 10a, most of the points lie on the diagonal line, revealing a good calibration model with relatively little noise. In Figure 10b, non-linearity can be detected, whilst Figure 10c shows a biased plot, with all the points lying on the same side of the diagonal. In Figure 10d, high levels of noise cause interference and deviation from the diagonal, while in Figure 10e, heteroscedastic noise causes deviation from the diagonal at certain measurements. Outliers in Figure 10f fit into the data range, but do not fit the model (Geladi, 2002).

E7. Interpretation of regression statistics

Several statistics can be generated to assess the goodness of a calibration model. Amongst these the correlation coefficient (r), the standard error of calibration (SEC), the standard error of prediction (SEP) and the F-statistic are important indicators. The square root of the coefficient of determination is simply called the correlation coefficient and describes the linear relationship between two variables (equation 8).

The correlation coefficient is a dimensionless measure of the degree to which calibration fits the data (Mark, 1992a; Osborne *et al.*, 1993). A value close to zero is an indication that the calibration is failing to relate the instrument readings to the reference values (Mark, 1992a). As the calibration coefficient increases, the instrument readings become better indications of the reference values. A correlation coefficient in excess of 0.9, preferably approaching 0.999, is ideal (Mark, 1992a; Wetzel, 1998). The calibration coefficient is, however, not an indication of how reliable the data is. It is therefore very important to take the SEP and the F-statistic into account when assessing a calibration. A correlation of less than 0.9 can be perfectly acceptable when the SEP resembles the SEL (Mark, 1992a; Osborne *et al.*, 1993; Wetzel, 1998).

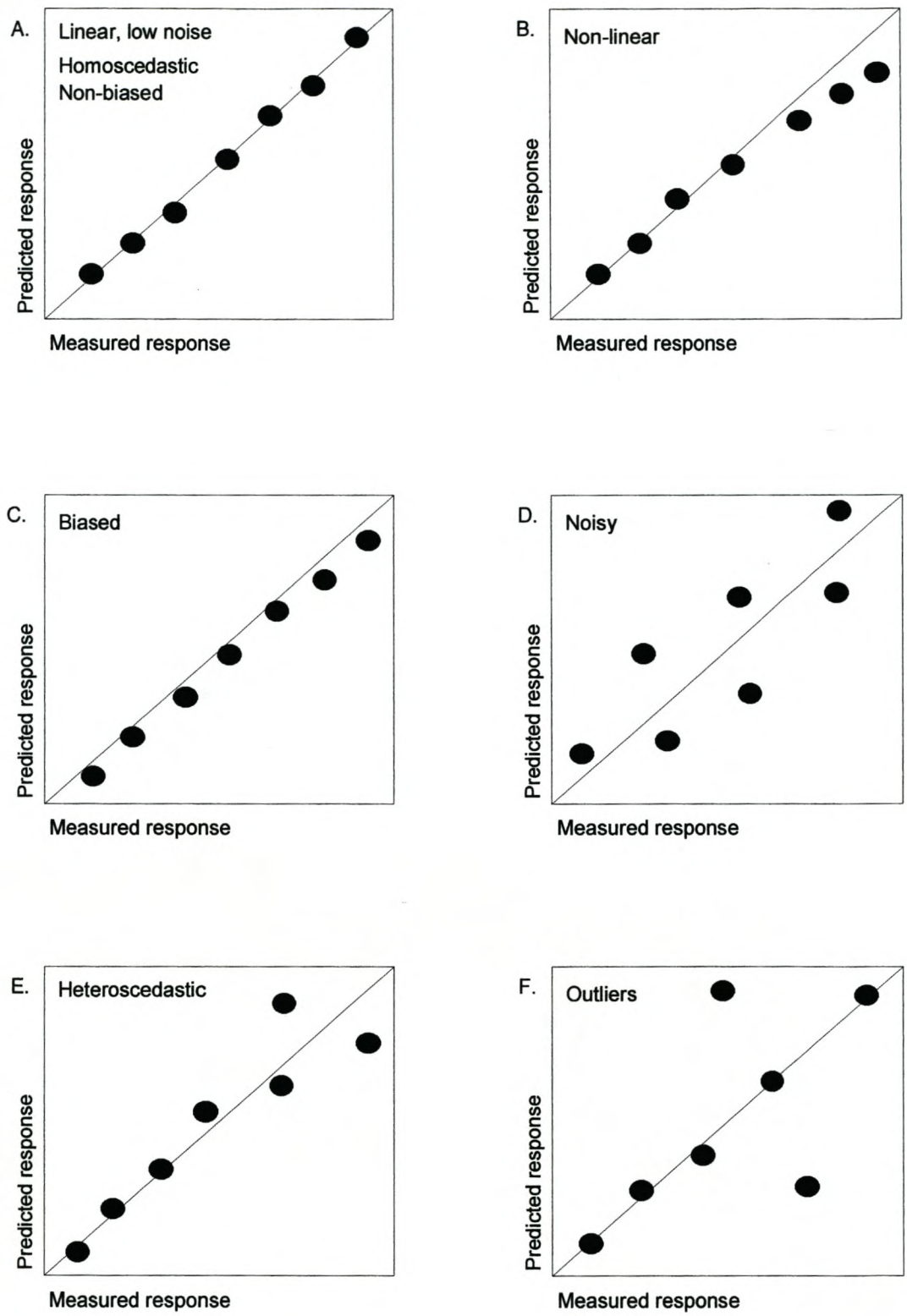


Figure 10. Different situations that may be encountered when comparing predicted and measured values in a test set (Geladi, 2002).

$$r = \frac{\sum_{i=1}^{n}\left(\hat{y}_i - \bar{y}\right)^2}{\sqrt{\sum_{i=1}^{n}\left(y_i - \bar{y}\right)^2}} \qquad \ldots\ldots\ldots\ldots 8$$

where y_i = the reference value for the i^{th} sample

$\hat{y}_i$ = the predicted value for the i^{th} sample

$\bar{y}$ = the mean y value for all the samples

n = the number of samples

The standard error of calibration (SEC) describes how well the calibration samples were fitted by the calibration equation. It is an indication of the standard error of difference between the constituent value from the calibration equation and the true constituent value obtained by the reference method (equation 9). The lower the SEC, the better the fit (Westerhaus, 1989a; Wetzel, 1998). A very low SEC, approaching zero can, however, be an indication of overfitting and implies that the model fits even the random measurement errors. In general, the SEC will exceed the standard error of laboratory (SEL), an indication of the reference method repeatability (equation 10), since the laboratory errors plus calibration (i.e. modelling) errors are incorporated into the prediction model (Westerhaus, 1989a).

$$\text{SEC} = \sqrt{\frac{1}{n-1-t}\sum_{i=1}^{n}\left(y_i - \hat{y}_i\right)^2} \qquad \ldots\ldots\ldots\ldots 9$$

where y_i = the reference value for the i^{th} sample

$\hat{y}_i$ = the predicted value for the i^{th} sample

n = the number of samples

t = the number of independent variables in the calibration equation

$$SEL = \sqrt{\frac{\sum\left(y_1 - y_2\right)^2}{2n}} \qquad \ldots\ldots\ldots\ldots 10$$

where y_1 and y_2 = results of duplicate determinations

n = the number of samples

The standard error of prediction (SEP) (equation 11) of the bias-corrected residuals (equation 13) is an indication of the performance or accuracy of the calibration equation on unknown samples from the same population (Westerhaus, 1989b). It gives an estimate of the magnitude of the error expected when independent samples are predicted using the model and allows for comparison between NIRS predicted values and reference data (Workman, 1992).

$$SEP \,/\, SECV = \sqrt{\frac{\sum_{i-1}^{n}\left(y_i - \hat{y}_i - Bias\right)^2}{n-1}} \qquad \ldots\ldots\ldots\ldots 11$$

where y_i = the reference value for the i^{th} sample

$\hat{y}_i$ = the predicted value for the i^{th} sample

n = the number of samples

The square root of the estimated squared prediction error (RMSEP) (equation 12) is measured in the same unit as *y* itself, rendering reference much easier (Martens & Næs, 1989). It is an estimate of the accuracy of the calibration against the reference method and is calculated using an independent test set.

$$RMSEP \,/\, RMSECV = \sqrt{\frac{\sum_{i-1}^{n}\left(y_i - \hat{y}_i\right)^2}{n}} \qquad \ldots\ldots\ldots\ldots 12$$

where y_i = the reference value for the i^{th} sample

$\hat{y}_i$ = the predicted value for the i^{th} sample

n = the number of samples

When prediction testing has been performed on an internal test set employing cross validation, the performance testing is also indicated by a standard error. In this case it is referred to as the standard error of cross validation (SECV) of the bias-corrected residuals, calculated using equation 11. Alternatively, the root mean square error of cross validation (RMSECV), which incorporates the bias effect (equation 12), can be used (Martens & Næs, 1989).

Various factors can influence the prediction ability of a model and knowledge of possible sources of prediction errors and how to limit them is essential to develop a robust calibration model. A model representing a bad fit to the data will produce a calibration equation without the ability to give precise results for future objects (Martens & Næs, 1989). Other error sources arise from calibration sets that do not cover the total range of variability in the population of future objects. Random noise arising from the instrument or calibration data can also lower the prediction ability of a model. This can, however, be counteracted by choosing a large number of calibration objects relative to the number of independent calibration parameters to be estimated (Martens & Næs, 1989).

Bias is a type of systematic prediction error and deserves special attention. Bias can be described as the difference between the expected value of $\hat{y}$ and y itself (equation 13) or the average absolute deviation from the mean y (Martens & Næs, 1989; Anonymous, 1997b). If the bias is near a value of zero, the overall error of validation (SEP) can be interpreted as the standard deviation of the NIR prediction.

$$\text{Bias} = \sum_{i-1}^{I}\left(\hat{y}_i - y_i\right)/I \qquad \text{.....13}$$

F. Advantages and limitations of NIR

NIRS has many advantages over conventional analytical methods but instead of competing, NIRS, chemical and other instrumental techniques often complement each other. High resolution and sensitive methods like HPLC and GC are quite

tedious and costly to perform, but deliver very accurate results. These methods should be employed to enhance the accuracy and precision of NIRS calibrations.

The low absorptivity of absorption bands in NIRS is compatible with moderately concentrated samples and longer path lengths compared with mid infrared analyses (Osborne *et al.*, 1993). This enables spectra to be measured by transmission through intact materials, which allows rapid and non-destructive analysis as no sample preparation is needed. Intact, opaque, biological samples can be analysed by diffuse reflectance, which makes NIRS a very simple technique to use and makes it the ideal application for on-line analyses.

The non-destructive nature of NIRS and lack of need for any chemical consumables, make NIRS a cost-effective method to employ in an environmentally conscious world where increasing disposal requirements for hazardous waste are installed for laboratories (Zoecklein *et al.*, 1994; Downey, 1998). The rapid nature of the technique, its ability to perform simultaneous analyses and analyses on high-moisture samples, add to the popularity of this method (Wetzel, 1998).

The major limitations of NIRS as an analytical technique, involves its dependence on other chemical methods of analyses that are sometimes less precise and equally empirical (Osborne *et al.*, 1993). A large data set incorporating large variation, which is often difficult to obtain, is essential to build a robust calibration (Wetzel, 1998). The initial high cost of the instrumentation is also an important factor when considering such a capital investment in a routine laboratory. The running cost of the equipment compared to the cost of consumables used in conventional chemical methods should, however, be made to assess the long-term financial implications and benefits of such an investment.

G. Infrared and near infrared applications in the wine and distillation industry

The applications of near infrared spectroscopy in the food and beverage industries are varied and include quantification of food ingredients and composites as reviewed by Williams & Stevenson (1990), Osborne *et al.* (1993), Iwamoto *et al.* (1995) and Wetzel (1998); food adulteration and authenticity as reviewed by Downey (1995 & 1996); the detection of external and internal defects in foods and crops and investigation of raw materials as reviewed by Osborne *et al.* (1993); and qualitative and sensory determinations as reviewed by Osborne *et al.* (1993), Downey (1995 & 1998) and Wetzel (1998).

In the wine industry, near infrared spectroscopy has found considerable use in various applications concerning wine analyses and qualitative determinations (Van de Voort, 1992). Kaffka & Norris (1976) and Baumgarten (1987) described an NIRS method for the determination of alcohol in wine and subsequent studies confirmed the suitability of NIRS for alcohol determinations. Near infrared spectroscopy delivered very satisfactory results in a survey of five methods for the analyses of alcohol strength in wine (Sneyd *et al.*, 1989). Van den Berg *et al.* (1997) reported on the feasibility of NIRS as an analytical technique to determine the alcohol concentration in alcoholic beverages without employing any sample pretreatment.

NIRS has been proposed as a technique for simultaneous measurement of grape colour, red pigment concentration analyses, °Brix and pH readings (Cope, 2000). Esler *et al.* (2002) successfully developed rapid prediction models for the total anthocyanins concentration, total soluble solids and pH in red wine grapes. Near infrared reflectance spectroscopy proved to be very successful in the simultaneous determination of ethanol, fructose and residual sugars in botrytized-grape sweet wines (Garcia-Jares & Médina, 1997). Glycerol and glucose quantification did, however, show great variation with the NIRS method. Burns (1994) proposed the employment of NIRS as a future analytical technique for determinations of total phenolic in wines. The compositional quality of grape, wine and spirits determined by means of scanning near infrared spectroscopy, were investigated by The Australian Wine Research Institute (Gishen & Dambergs, 1998; Cope, 2000). Quality indicators investigated during the trial included colour, °Brix, total phenolics and the concentration of glycosyl-glucose in whole grapes, grade assessment ("in-house" dollar value) for table wines and methanol content of spirits. The preliminary evaluation of the applicability of near infrared spectroscopy to determine compositional quality showed considerable promise with potential for immediate application in the wine industry.

Dambergs *et al.* (2001), explored the potential of NIRS as a tool to predict wine sensory quality. In South Africa, Manley *et al.* (2001) used FT-NIRS to measure the percentage of sugar in must and to discriminate between different must samples in terms of their free amino nitrogen values. It also proved to be a rapid method to discriminate between Chardonnay wine samples in terms of their malolactic fermentation status. Table wines were also successfully discriminated in terms of their ethyl carbamate content. Discrimination between different tartrates used in the

wine industry was successfully performed with FT-NIRS (Anonymous, 1998). Kaffka & Norris (1976) determined the tartaric acid content in wine.

Spectroscopy dealing more with the mid-infrared region has also found classification applications in the wine industry. Edelmann *et al.* (2001), employed mid-infrared spectroscopy combined with multivariate data analysis to successfully classify Austrian red wines in terms of their phenol contents. Schindler *et al.* (1998) developed a rapid, automated method for the simultaneous detection of sugars, alcohols and organic acids in wine. Multivariate analysis in association with different quality control methods including chromatography and infrared spectroscopy have been employed successfully for the assessment of wine authenticity (Arvanitoyannis *et al.*, 1999). Gishen & Holdstock (2000) assessed the Foss winescan FT 120 instrument (a Fourier transform infrared spectrophotometer) for the simultaneous determination of several wine components and found that the instrument could successfully predict ethanol, glucose/fructose, pH, total acid and volatile acid in wine. Poor predictions were obtained for total and free sulphur dioxide in wine. Coimbra *et al.*, (2002) developed a Fourier transform infrared method to discriminate between different white wine polysaccharide extracts and the quantification of mannose in Portuguese wine samples.

H. Conclusions

A global need exists for rapid, non-destructive methods that can be used for routine analysis in all fields of food and beverage production. Various types of analyses are carried out as an essential part of process and quality control in laboratories. The distillation industry is no exception to this rule. Legislation and process control all set strict requirements to which input materials must comply. Near infrared spectroscopy is an alternative method with considerable potential that can be applied to rapidly solve various analytical problems in the distillation industry specifically. It is of the utmost importance, however, to thoroughly understand the fundamentals of near infrared spectroscopy. Like all analytical methods, it has limitations but through constant research and innovation, the capabilities can be extended to maximise the performance of this very exciting technology. NIRS is dependent on other accurate methods to enhance its own performance. Rather than compete with other sensitive analytical procedures like HPLC and GC, these methods should complement each other.

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CHAPTER 3

QUANTIFICATION OF THE QUALITY PARAMETERS OF DISTILLING WINE BY NEAR INFRARED SPECTROSCOPY

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Summary

Fourier transform near infrared spectroscopy (FT-NIRS) was investigated as a rapid screening method for the determination of the alcohol, volatile acid and total sulphur dioxide contents of distilling wine. Rapid assessment of these parameters is needed to aid the selection process of suitable wines for grape spirit distillation. For each of the parameters, PLS models were developed and calibrations derived using The Unscrambler (ver. 6.11). The wine samples were presented in two different path length quartz cuvettes, 0.2 mm and 1 mm. Independent validation was used to assess the prediction abilities of the models. The best results for all three parameters were obtained with the sample set analysed in the 1 mm path length cuvette. Strong correlations were found between the near infrared spectral data and the reference data of the distilling wine and resulted in excellent predictions for the alcohol content ($r = 0.99$, SEP = 0.18% v/v, RER = 30 for the 1 mm cuvette measurements; $r = 0.97$, SEP = 0.27% v/v, RER = 21 for the 0.2 mm cuvette measurements). The influence of path length on these measurements was statistically insignificant ($p \geq 0.05$). Volatile acid and total sulphur dioxide delivered unsuccessful prediction results. A poor prediction performance was found for the volatile acid quantification in distilling wine and the best correlation of prediction was 0.67 with a high SEP of 0.33 $g.L^{-1}$ measured in the 1 mm cuvette. A second dataset with a narrow concentration range (0.1 – 1.8 $g.L^{-1}$) resulted in a lower correlation for the 1 mm cuvette measurements ($r = 0.60$), but a more acceptable SEP of 0.26 $g.L^{-1}$ was obtained. An improvement in the prediction could not be repeated for the 0.2 mm cuvette measurements. The accuracy of the predictions (RER < 10) was not acceptable for future analytical measurements. Analysis of variance (ANOVA) found the differences in predicted values of volatile acid between the 1 mm and 0.2 mm measurements to be statistically significant over the dataset. The total sulphur dioxide delivered unsuccessful prediction results with no significant difference

between the 0.2 mm and 1 mm cuvette measurements. The best regression results for all three components were obtained over the near infrared region between 650 nm and 2500 nm.

Introduction

Distilling wine is produced from grape juice obtained during wine production with any wine grape cultivar (Steger, 2001). Juice retrieved from the first pressing of grapes during the pressing season is used for table wine fermentation. The juice from the second and third press is destined for distilling wine production. The distilling wine juice is fermented in a separate tank and juice obtained from poor quality grapes is sometimes included. Fermentation terminates when the residual sugar content is exhausted, resulting in a product with an alcohol content ranging between 6 and 13% v/v (Steger, 2001; Weitz, 2001).

Alcoholic strength, the volatile acidity and the total sulphur dioxide content are the basic parameters that serve as indicators of the quality of the wine. Distilling wine must therefore, contain a total sulphur dioxide level of less than 200 $mg.L^{-1}$ (parts per million), have an alcohol strength higher than 7.5% v/v and a volatile acidity of less than 1.2 $g.L^{-1}$ (Steger, 2001).

Alcohol serves as a basis of payment for distilling wine and is defined by legal limits in various types of alcoholic beverages. The alcohol content must be displayed on the labels of all alcoholic beverages (Burns, 1994; Anonymous, 1996). Acetic acid is the most significant volatile acid present in wine, acts as an indicator of microbial spoilage and plays an important organoleptic role (Burns, 1994; Zoecklein *et al.*, 1994; Anonymous, 1996). Sulphur dioxide is widely used in the wine industry as a chemical antioxidant and inhibitor of microbial activity (Zoecklein *et al.*, 1994). Only the free dissolved and the undissociated forms inhibit the growth or kill spoilage bacteria, fungi and wild yeasts (Anonymous, 1996; Ribéreau-Gayon *et al.*, 2000). Sulfites were historically generally regarded as safe, but the U.S. Food and Drug Administration (FDA) has determined that the presence of sulfites in food and beverages poses a health threat to a certain class of asthmatic individuals (Zoecklein *et al.*, 1994; Ribéreau-Gayon *et al.*, 2000). As a result, sulfites present in alcoholic beverages at levels exceeding 10 $mg.L^{-1}$ total sulphur dioxide, must be declared on labels. The control of sulphur dioxide in wines is also important as excess can impart an undesirable taste on the nose and palate (Anonymous, 1996; Steger, 2001).

To further enhance the quality of its products, the distillation industry has a need for simple, rapid and cost effective techniques to objectively evaluate the quality of the wines used to produce distilled beverages. Screening methods are used in routine laboratories to divide samples within a limited range from those that fall outside the permitted limits (Prichard *et al.*, 1995). Fast screening methods would be ideal to measure the parameters of importance for selection purposes. Screening methods must be extremely rapid, thus permitting a high throughput of samples at low cost (Prichard *et al.,* 1995). These methods can be qualitative or quantitative, and will be validated only to the extent of the limit of detection by the operational laboratory. Limit of detection indicates the lowest possible detectable concentration that can be measured by a method (Prichard *et al.*, 1995). Ideally, the limit of detection of the method selected should be at least one-tenth of the concentration to be measured.

NIRS can possibly be applied as a rapid and reliable method to aid in the selection of suitable wines for distillation purposes. NIRS methods have gained considerable recognition, given increasing disposal requirements for hazardous waste generated by laboratories. Once calibrated, NIRS uses no reagents and consequently generates no waste (Zoecklein *et al.,* 1994).

The measurement of the alcohol concentration in wine and alcoholic beverages using NIRS has been well established (Kaffka & Norris, 1976; Baumgarten, 1987; Sneyd *et al.*, 1989; Garcia-Jares & Médina, 1997; Van den Berg *et al.*, 1997). Any determination of alcohol or phenols is expected to work because OH absorptions are strong in the near infrared region (Wetzel, 1998).

Overtone bands of the carboxyl group found in carboxylic acids (organic acids) have been observed in the region of 1900 nm (Osborne *et al.*, 1993). Kaffka & Norris (1976) determined the tartaric acid content in wine using selected interference filters. Total acid, volatile acid and pH in wine have been predicted successfully with mid-infrared spectroscopy using the Foss Winescan FT 120 (Foss Electric, Denmark) (Gishen & Holdstock, 2000).

It has been possible to determine some inorganic constituents indirectly either by way of some organic moiety to which they are bound or by virtue of some effect on some absorption band (Osborne *et al.*, 1993). Gishen & Holdstock (2000) attempted total sulphur dioxide prediction with the Foss Winescan, a mid-infrared

predictive instrument. A reasonable correlation (r = 0.83) was obtained but the prediction error was unacceptably high (SEP = 23 $mg.L^{-1}$).

Spectral data acquisition in liquids is performed through transmittance, where the incident light illuminates one side of the sample and the unabsorbed portion of the incident light is channelled to the detector on the opposite side (Kawano, 2002). In transmittance measurements, the entire path length of a sample is integrated into the spectral measurement (Workman & Burns, 1992). The cuvette width of a flow-through cell can be adjusted to optimise the absorption sensitivity. It is important to consider temperature changes; therefore, a controlled environment is desirable. The path length of the cell depends on the wavelength region and samples used (Kawano, 2002).

Currently the alcohol strength, volatile acidity and total sulphur dioxide levels in distilling wine are measured using time-consuming or labour-intensive analytical methods. These methods include distillation combined with pycnometry, refractometry or dichromate oxidation for alcohol, steam distillation for volatile acid assays and the aeration method for total sulphur dioxide which requires the distillation of the acidified sample solution into a hydrogen trap or the Ripper method, though simple to perform, universally recognised not to be very sensitive. NIRS could potentially replace some of these methods to perform simultaneous analysis to assess the quality of the wine before purchasing from the producers and prior the advancement of the distillation process.

Objective

The objective of this study was to assess the suitability of FT-NIRS for the determination of alcohol, volatile acidity and total sulphur dioxide contents in turbid distilling wine samples. The study also aimed to look at the effect of path length as induced by two different path length cuvettes during NIRS measurements.

Materials and methods

Wine samples

A selection of 108 distilling wine samples, all originating from the Western Cape region and distributed throughout the wine season of 2002, were collected from Distell in Worcester, South Africa. The distilling wine samples were analysed on receipt with conventional analytical methods and stored at 4°C. The samples were

allowed to equilibrate to room temperature before recording the near infrared absorbencies with a Fourier transform near infrared spectrophotometer.

Chemical analyses

Alcohol

Nearly all the alcohol present in grape wine is ethanol (ethyl alcohol) with a boiling point of 78.37°C (Anonymous, 1996). The alcohol content of the wine was determined pycnometrically by measuring the specific gravity of the wine and the alcohol-water distillate as described by the AOAC, method 920.57 (AOAC 2000).

Volatile acidity

Steam distillation of the sample as described by Gowans (1964), was followed by titration with standardised sodium hydroxide to a phenolphthalein end point and the results reported as acetic acid in $g.L^{-1}$ (AOAC, 2000).

Total sulphur dioxide

The sulphur dioxide content of the brandy base wine was determined iodometrically by potassium iodate/iodide using the AOAC Ripper method 940.20. (Anonymous, 1996; AOAC 2000).

Fourier transform near infrared spectroscopy (FT-NIRS) measurements

Near infrared spectra were recorded in transmittance mode at 2 nm intervals using a Perkin-Elmer Spectrum Identicheck™ 2.0 FT-NIR system (Perkin-Elmer corp., Norwalk, CT., U.S.A.). The spectra were collected between 650 and 2500 nm, with a 16 scan sequence at a resolution of 8 cm^{-1}. The liquid samples were presented in a 1 mm path length Quartz UV/VIS spectroscopy cuvette (Perkin-Elmer) as well as in a 0.2 mm Quartz cuvette (Helma).

Chemometric operation

Spectra were exported from the Perkin-Elmer *.sp format as ASCII files and converted via a macro reader into The Unscrambler ver. 6.11 software (CAMO AS, Trondheim, Norway). Light scattering and density variations cause baseline fluctuations in transmittance spectra (Wetzel, 1998; Fearn, 1999). Multiplicative scatter correction (MSC) was applied to the spectra to eliminate baseline fluctuations.

Second derivative spectra are powerful in removing additive and multiplicative baseline variations of the spectra (Wetzel, 1998; Fearn, 1999). Second derivatives were calculated using a nine point, second order Savitzky-Golay filter. The partial least squares (PLS) algorithm was used to relate the spectral and chemical data into bilinear models. Different spectral regions and concentration ranges within each dataset were examined to determine the optimum conditions for each components' prediction modelling.

The square root of the coefficient of determination, simply called the correlation coefficient (r) was used to describe the linear relationship between the chemical measurements and the NIRS predicted values (equation 1).

$$r = \frac{\sum_{i=1}^{n}\left(\hat{y}_i - \bar{y}\right)^2}{\sqrt{\sum_{i=1}^{n}\left(y_i - \bar{y}\right)^2}} \qquad \text{.....1}$$

where y_i = the reference value for the i^{th} sample

$\hat{y}_i$ = the predicted value for the i^{th} sample

$\bar{y}$ = the mean *y* value for all the samples

n = the number of samples

The standard error of laboratory (SEL) is an indication of the reference method repeatability (equation 2) and can also be referred to as the precision of the conventional chemical analysis (Westerhaus, 1989). The comparison between the SEL of a reference method and the SEP of the NIRS method is used to assess the performance of NIRS. A chemical reference method is used to develop an NIRS calibration; the SEP of NIRS can therefore not be lower than the SEL of the reference method.

$$SEL = \sqrt{\frac{\sum\left(y_1 - y_2\right)^2}{2n}} \qquad \text{.....2}$$

where y_1 and y_2 = results of duplicate determinations

n = the number of samples

Independent validation with 35 randomly chosen samples (*ca.* 33%) was also performed to test the prediction performance of the models on a set of samples not present in the calibration. The standard error of prediction (SEP) of the bias-corrected residuals (equation 3) was used to express the accuracy of the calibration. The square root of the estimated squared prediction error (RMSEP) (equation 4) was also measured as an indication of the accuracy of the calibration, but with consideration of the bias effect. Bias (equation 5) has a strong random component that can change for one validation set to another and needs to be considered. The bias is interpreted as the average difference between $\hat{y}$ and y in the prediction set (Næs & Isaksson, 1992).

$$SEP = \sqrt{\frac{\sum_{i-1}^{n}\left(y_i - \hat{y}_i - Bias\right)^2}{n-1}} \qquad \text{.....3}$$

$$RMSEP = \sqrt{\frac{\sum_{i-1}^{n}\left(y_i - \hat{y}_i\right)^2}{n}} \qquad \text{.....4}$$

$$Bias = \frac{1}{n}\sum_{i-1}^{n}\left(y_i - \hat{y}_i\right) \qquad \text{.....5}$$

where y_i = the reference value for the i^{th} sample

$\hat{y}_i$ = the predicted value for the i^{th} sample

n = the number of samples

The prediction performance of the NIRS models as related to the range of the chemical measurements, were lastly evaluated with the range over error ratio (RER statistic) (Williams & Sobering, 1996). The RER statistic is the ratio of the range of the reference data for the validation samples to the SEP or SECV. The range of the

dataset and therefore the variance in the reference original reference data was limited. The RER value should ideally be at least 10, indicating that the equation is capable of predicting the required values with an accuracy of at least one tenth of the range.

Analysis of variance

Analysis of variance (ANOVA) was performed with the Statistica ver. 6.00 software package (Stat-Soft, Tulsa, OK., U.S.A.) on the predicted NIRS values obtained for the validation samples.

Results and discussion

The effect of path length on the measurement of the NIRS absorbance spectra of distilling wine can be seen in Figures 1 and 2 respectively. Absorption maxima are clearly evident at 1455, 1825, 1933 and 2265 nm for the measurements in both the 0.2 mm and 1 mm path length cell. The first overtone water bands can be seen at 1933 nm. This is the most well known of all absorptions in the near infrared region and is due to the combination of OH stretch and OH bend vibrations of the water molecule. The second overtone water band is visible at 1455 nm. The band around 2265 nm is a combination of CH stretching and CH bending vibrations of the ethanol molecule. The second derivative spectra also clearly show the CH combination band at 2265 nm.

Possible detector saturation could be responsible for the flattened, broad peak seen between 1900 and 2000 nm in the 1 mm path length absorption spectrum. PCA plots studied in conjunction with reference data values were employed as outlier detection strategy.

Alcohol

It was found that a very strong correlation existed in the data set for the FT-NIRS predictions of alcohol in distilling wine. A summary of the best regression results is given in Table 1 for the 0.2 mm and 1 mm path length measurements. The correlation was strong for both the 0.2 mm cell measurements ($r = 0.97$) as shown in Figure 3 and for the 1 mm measurements ($r = 0.99$) as shown in Figure 4. A better prediction was obtained with the 1 mm cell as a lower SEP (0.18% v/v) compared to

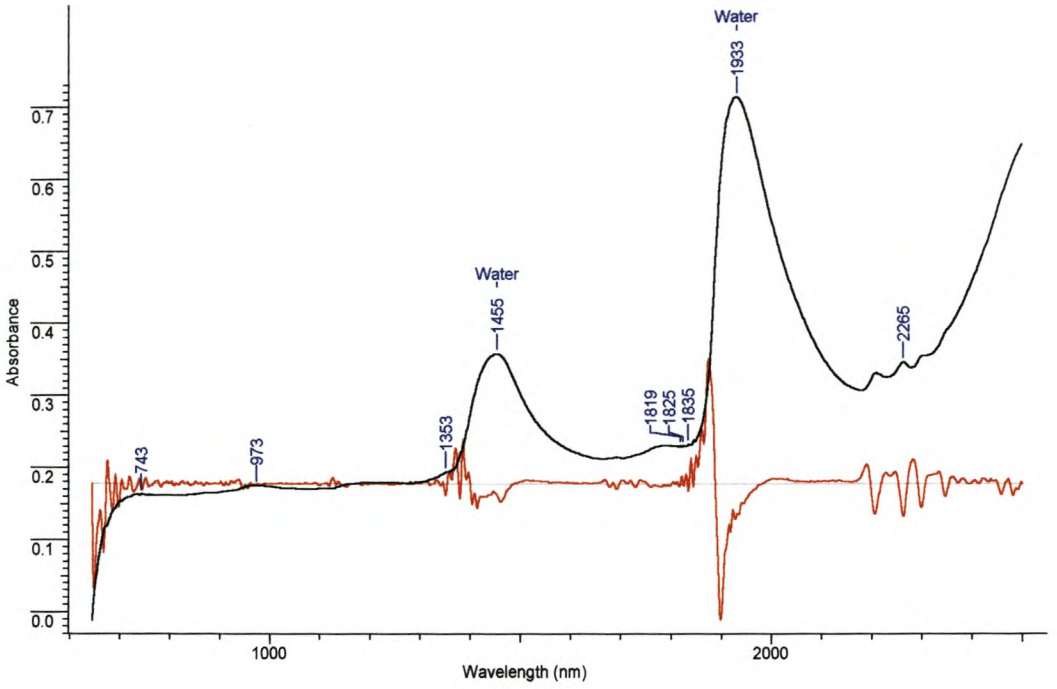

Figure 1. Absorption spectrum of distilling wine (black line) measured in a 0.2 mm quartz cuvette and its second derivative spectrum (red line).

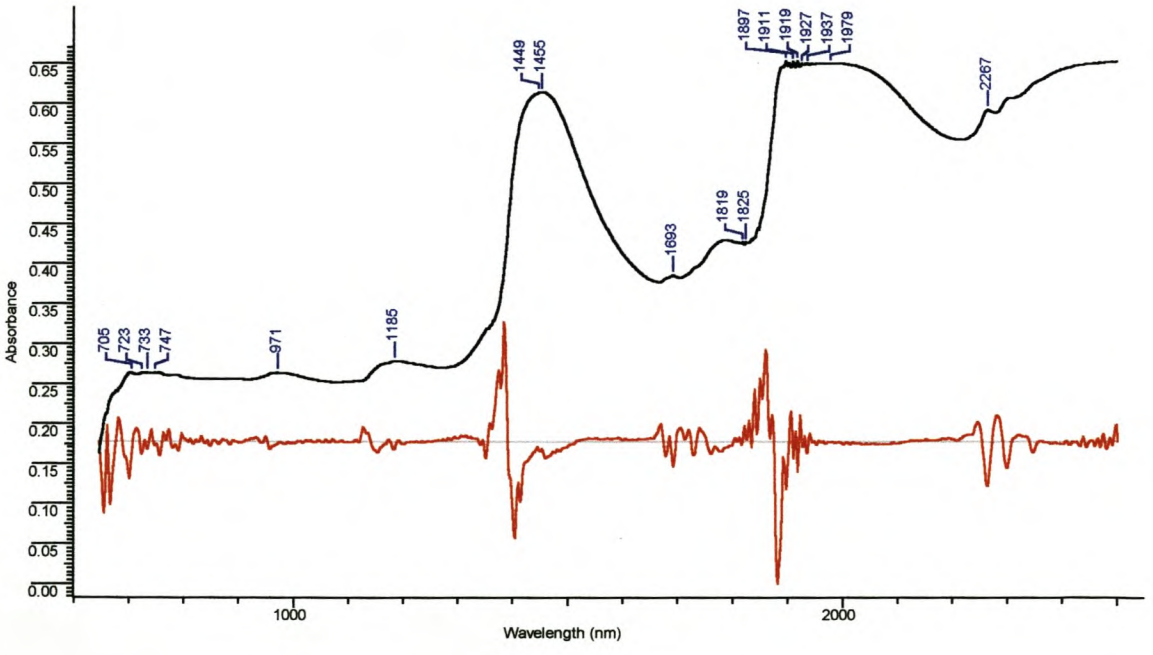

Figure 2. Absorption spectrum of distilling wine (black line) measured in a 1 mm quartz cuvette and its second derivative spectrum (red line).

the SEP of 0.27% v/v for the 0.2 mm cell were obtained.

The issue of detector saturation between 1900 and 2100 nm with the 1 mm path length measurements is raised in the absorption plot in Figure 2. The regression coefficient plot in Figure 5, however, indicates the significant contribution of each wavelength to the alcohol modelling of the 1 mm spectral measurements. The wavelength range between 1900 and 2100 nm contributed very little towards the variability in the data. The large deviations from zero in the region of 2265 nm in Figure 5 show that this specific area contributed significantly to the variability in the data. This plot therefore confirms that alcohol was indeed modelled in this instance.

Analysis of variance (ANOVA) performed on the predicted values of the the two calibrations, however, indicated that the 1 mm measurements did not predict significantly better ($p>0.05$) than the 0.2 mm for the alcohol content (Figure 6). The strong correlation for alcohol was expected given that the measurement of alcohol in wine and alcoholic beverages have been well established (Kaffka & Norris, 1976; Baumgarten, 1987; Sneyd *et al.*, 1989; Garcia-Jares & Médina, 1997; Van den Berg *et al.*, 1997) and the OH bond of alcohol has a strong near infrared absorbance capacity.

The standard error of prediction obtained for the regression model was high compared to the SEL of 0.1% v/v for the reference measurement. This could be attributed to the typical Gaussian distribution of samples along the analyte range in frequency of occurrence, as shown in Figure 7. The models were therefore able to predict samples alcohol with values close to the mean better than samples with values at the extreme ends of the dataset.

The RER values (RER > 10) indicated that the accuracy of the equations were acceptable and the SEP of the models reported in this work are sufficiently low to recommend FT-NIRS as an accurate analytical method for the alcohol concentration measurement in distilling wine. The best correlation was obtained when the entire spectra (650 nm – 2500 nm) and concentration range (7.7 – 13.4% v/v/) were incorporated into the regression modelling.

Table 1. Summary of the independent validation statistical results obtained for alcohol (% v/v) in distilling wine.

	0.2 mm	1 mm
Range	7.7 – 13.4	7.7 – 13.4
Mean	10.5	10.5
n_{cal}	72	72
n_{val}	35	35
r	0.97	0.99
SEP	0.27	0.19
RMSEP	0.35	0.18
Bias	0.228	-0.079
Nr. of PLS factors	7	9
RER	21	30

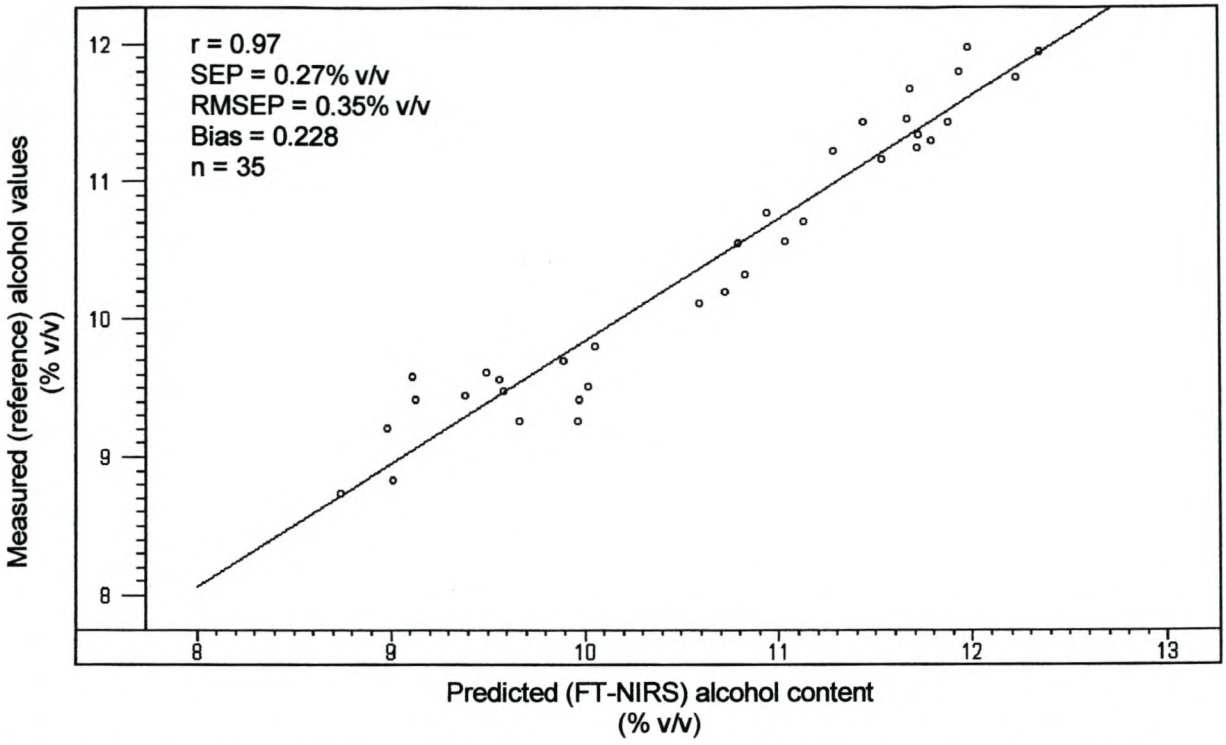

Figure 3. Plot of the predicted versus the measured alcohol concentrations (% v/v) for the distilling wine samples measured in a 0.2 mm cuvette.

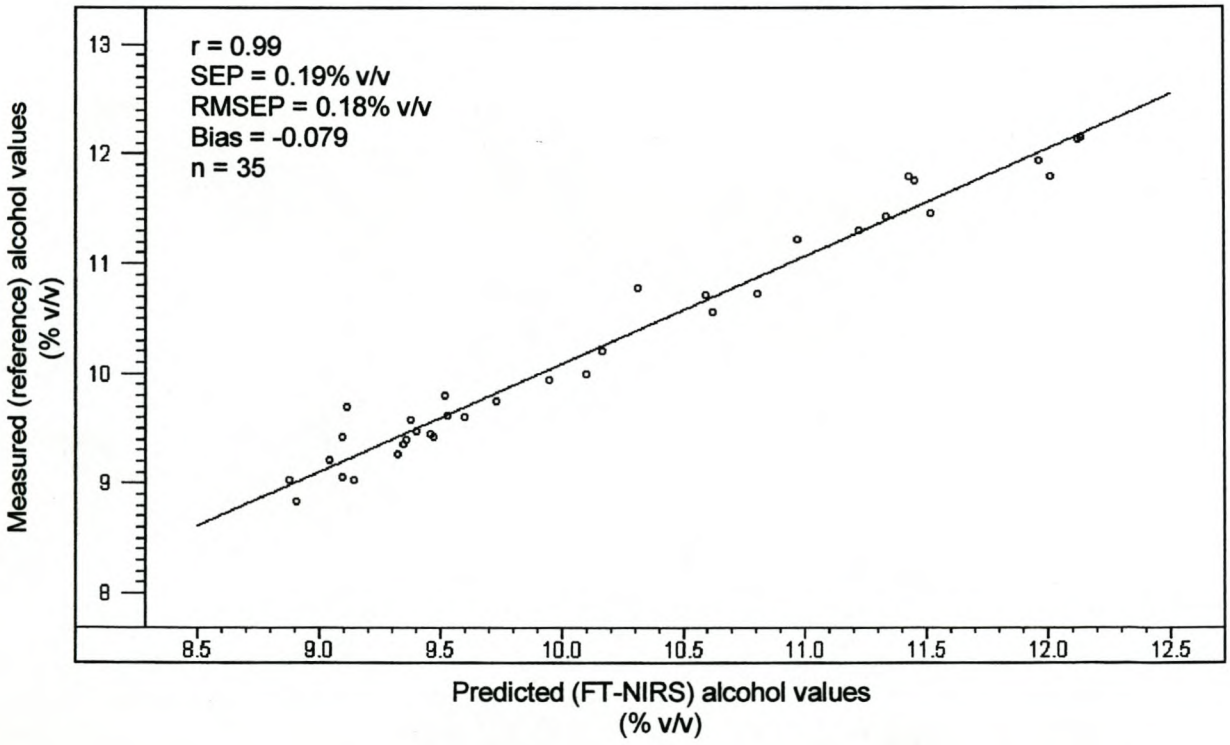

Figure 4. Plot of the predicted versus the measured alcohol concentrations (% v/v) for the distilling wine samples measured in a 1 mm cuvette.

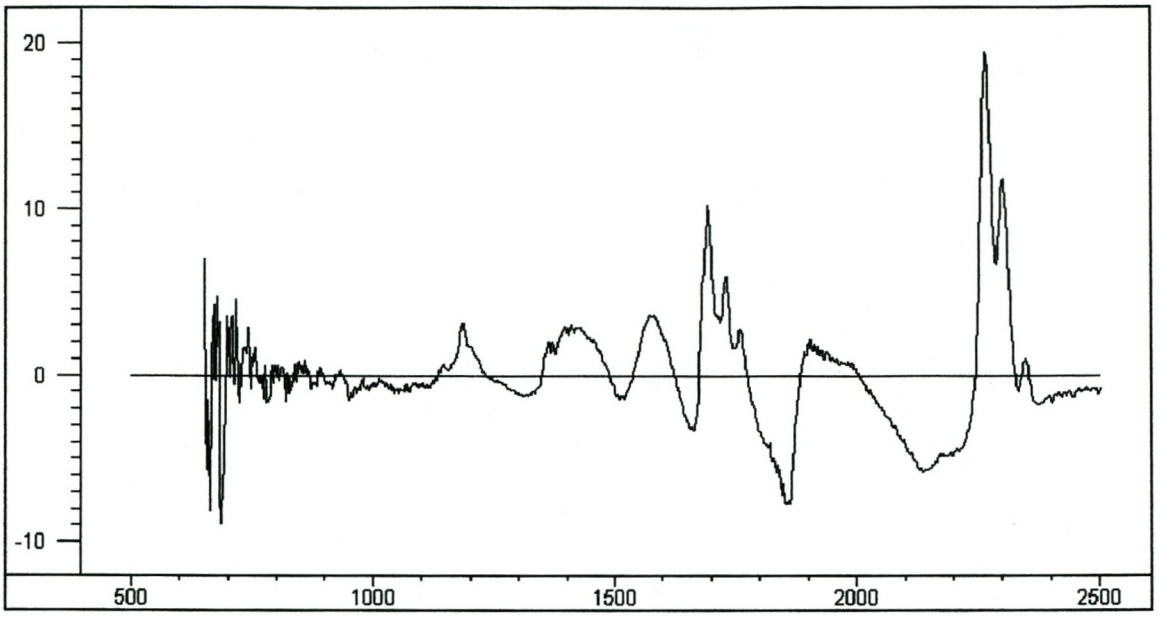

Figure 5. Regression coefficient plot of the 1 mm distilling wine spectral measurements regressed against the alcohol reference data using 9 PLS factors.

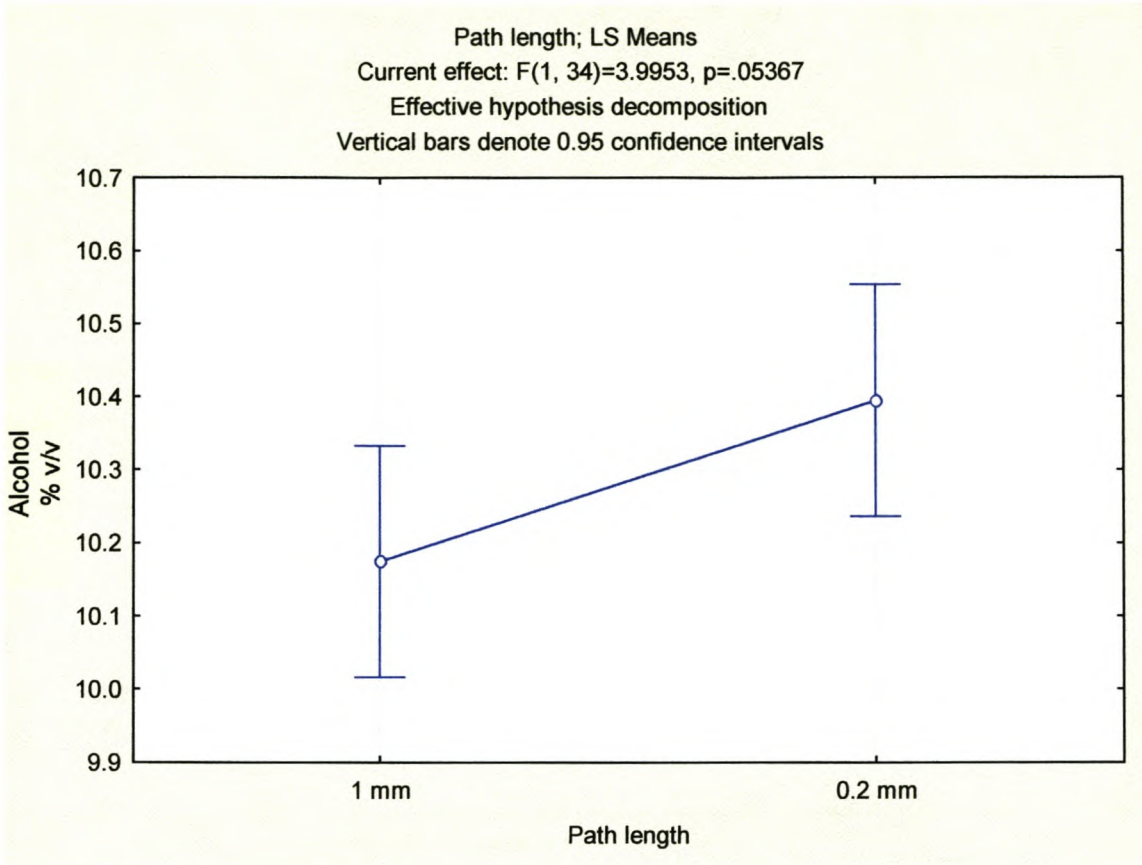

Figure 6. The ANOVA plot of the predicted values for the alcohol concentrations (% v/v) in distilling wine for measurements in a 1 mm and a 0.2 mm cuvette.

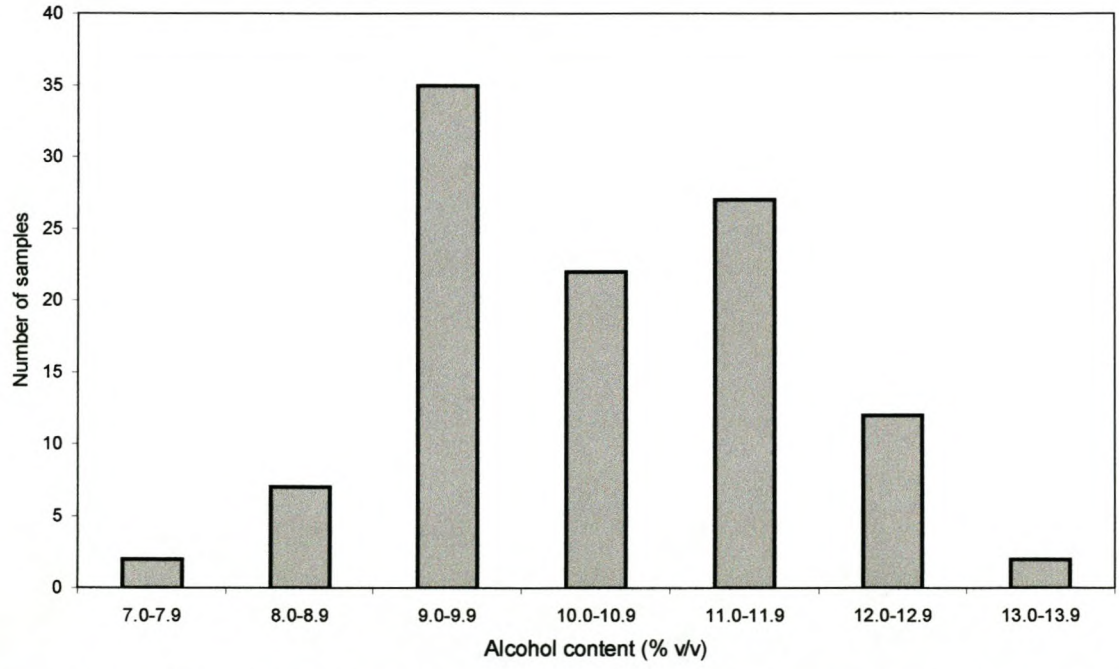

Figure 7. The distribution of samples along the alcohol concentration range in the distilling wine sample set.

Volatile acidity

Three samples in the dataset were identified as spectral outliers during principal component analysis of the data. These samples were extremely turbid and due to their interference with the calibration performance, discarded from the dataset. The results of the independent validation that were obtained during the attempted prediction of volatile acid in distilling wine are shown in Table 2. The correlation between the spectral measurements and the volatile acid concentrations was rather poor for both the 0.2 mm cell measurements ($r = 0.60$) as shown in Figure 8 and for the 1 mm measurements ($r = 0.71$) as shown in Figure 9. A slightly better prediction resulted with the 1 mm cell as a lower SEP (0.34 $g.L^{-1}$), compared to the SEP of 0.39 $g.L^{-1}$ for the 0.2 mm cell, was obtained. It became clear from the correlation graphs in Figures 8 and 9, that the models followed a heteroscedastic pattern at the extreme ends of the concentration range.

New calibration models were developed that excluded samples with a volatile acid content exceeding 2 $g.L^{-1}$, and these were also tested on an independent validation set. The results for the 0.2 mm and 1 mm measurements are shown in Table 3. The correlation of prediction for both measurements deteriorated rather than improved as can be seen in Figure 10 for the 0.2 mm measurements and Figure 11 for the 1 mm measurements. There was, however, a slight improvement in the prediction error for both the 0.2 mm (SEP = 0.31 $g.L^{-1}$) and 1 mm (SEP = 0.25 $g.L^{-1}$). The prediction error of all the models were high compared to the accepted SEL of the reference method (0.05 $g.L^{-1}$) and the accuracy of the predictions ($RER < 10$) indicated that the models were not suitable for prediction testing on future samples.

ANOVA performed on the predicted values of the two calibrations also indicated that the 1 mm measurements did predict significantly better ($P \leq 0.05$) than the 0.2 mm for the volatile acid content of distilling wine, as can be seen in Figure 12. In transmittance measurements, the entire path length of a sample is integrated into the spectral measurement. As a result, compounds present in lower concentrations in a liquid matrix, will be detected better in a cell with a longer path length.

The distribution pattern of the volatile acid concentration range in the distilling wine sample set is shown in Figure 13. This presented a typical problem associated with NIRS calibration, as the levering effect of a few samples at either end of the range were counteracted by the large number of samples in the centre. The best

correlation was obtained when the entire spectra (650 nm – 2500 nm) were incorporated into the regression modelling.

Table 2. Summary of the independent validation statistical results obtained for volatile acidity ($g.L^{-1}$) in distilling wine in the range 0.1-2.7 $g.L^{-1}$.

	0.2 mm	**1 mm**
Range	0.1 – 2.7	0.1 – 2.7
Mean	0.9	0.9
n_{cal}	70	70
n_{val}	34	34
r	0.60	0.67
SEP	0.36	0.33
RMSEP	0.38	0.33
Bias	0.135	-0.027
Nr. of PLS factors	5	9
RER	7.2	7.9

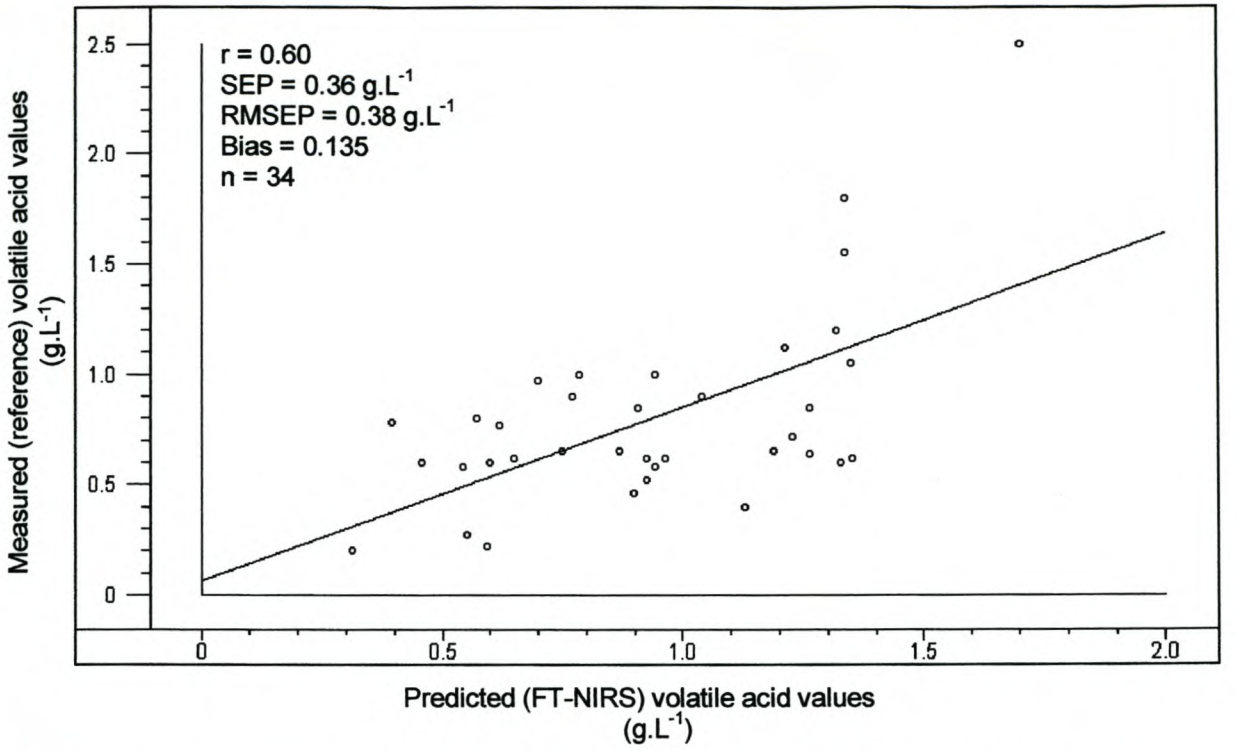

Figure 8. Plot of the predicted versus the measured volatile acidity ($g.L^{-1}$) for the distilling wine samples measured in a 0.2 mm cuvette in the range between 0.1-2.7 $g.L^{-1}$.

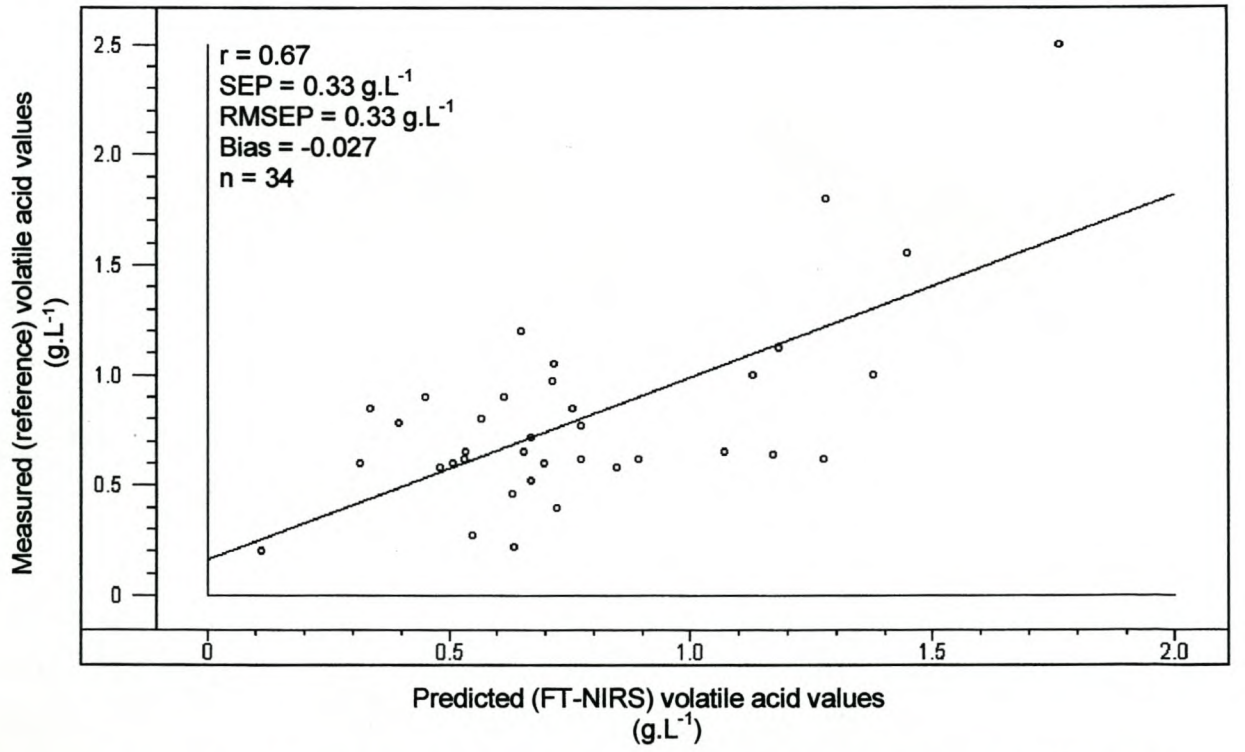

Figure 9. Plot of the predicted versus the measured volatile acidity ($g.L^{-1}$) for the distilling wine samples measured in a 1 mm cuvette in the range between 0.1-2.7 $g.L^{-1}$.

Table 3. Summary of the independent validation statistical results obtained for volatile acidity in distilling wine in the concentration range 0.1-1.8 $g.L^{-1}$.

	0.2 mm	**1 mm**
Range	0.1 –1.8	0.1 – 1.8
Mean	0.8	0.8
n_{cal}	67	67
n_{val}	32	32
SEP	0.32	0.26
RMSEP	0.32	0.26
Bias	-0.035	0.049
r	0.21	0.60
Nr. of PLS factors	3	8
RER	5.3	6.5

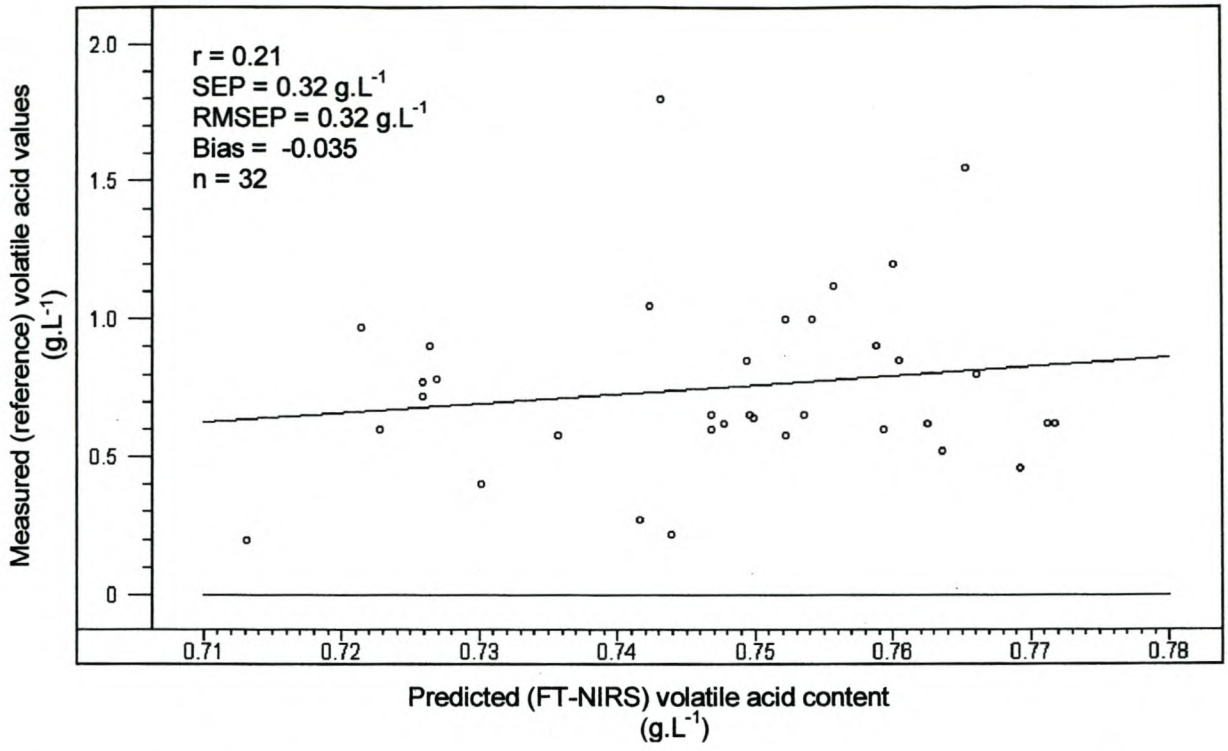

Figure 10. Plot of the predicted versus the measured volatile acidity ($g.L^{-1}$) for the distilling wine samples measured in a 0.2 mm cuvette in the range 0.1-1.8 $g.L^{-1}$.

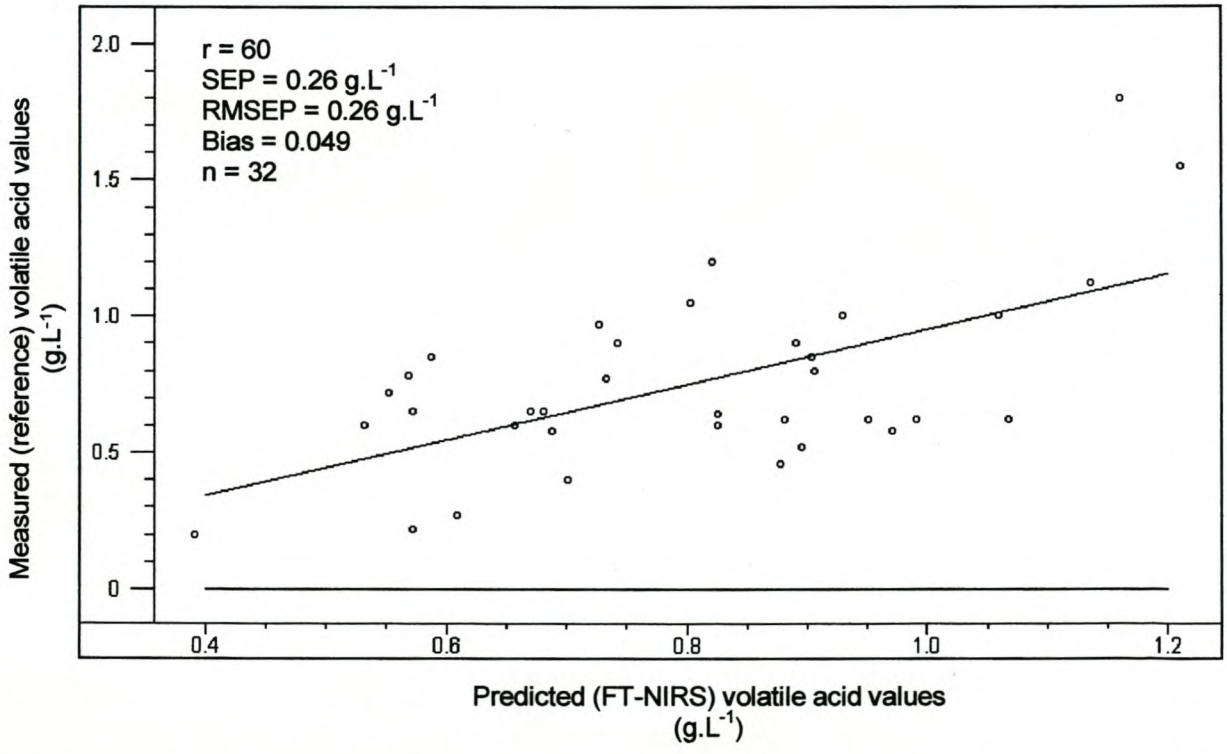

Figure 11. Plot of the predicted versus the measured volatile acidity ($g.L^{-1}$) for distilling wine samples measured in a 1 mm cuvette in the range 0.1-1.8 $g.L^{-1}$.

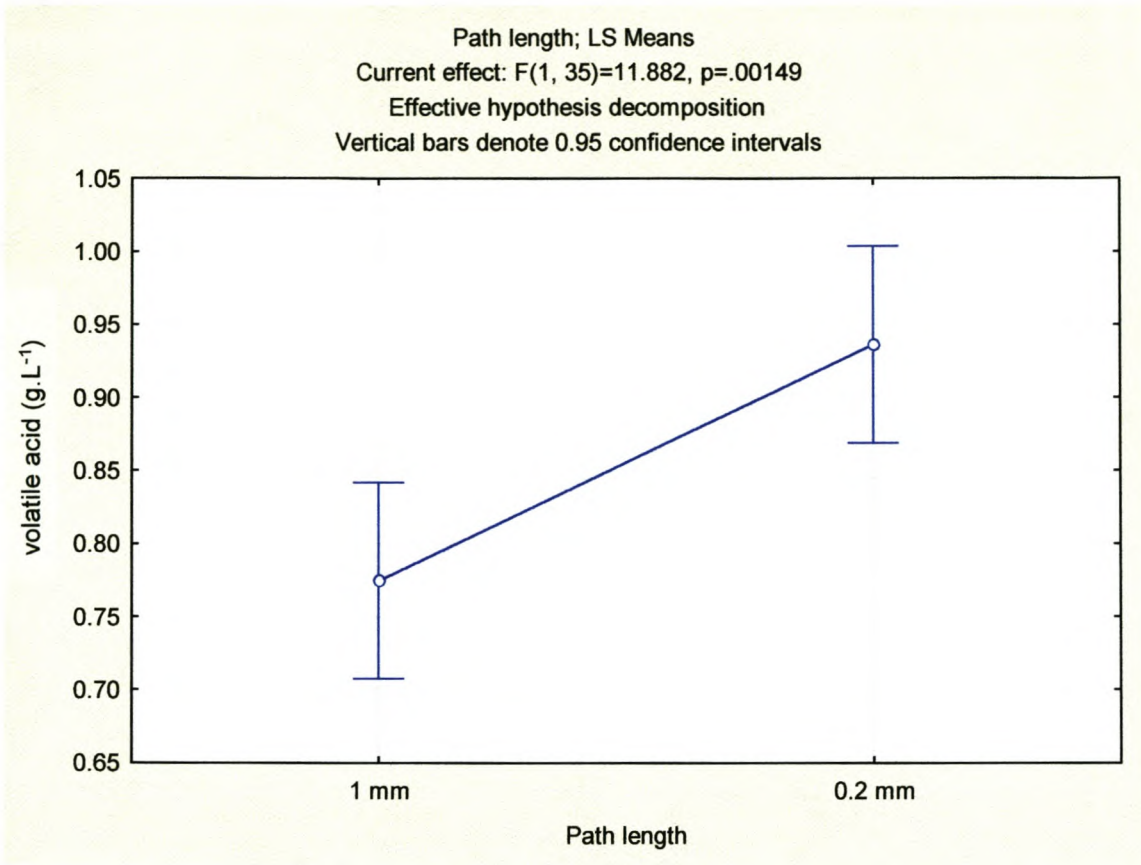

Figure 12. The ANOVA plot of the predicted values for the volatile acid content ($g.L^{-1}$) in distilling wine for measurements in a 1 mm and a 0.2 mm cuvette.

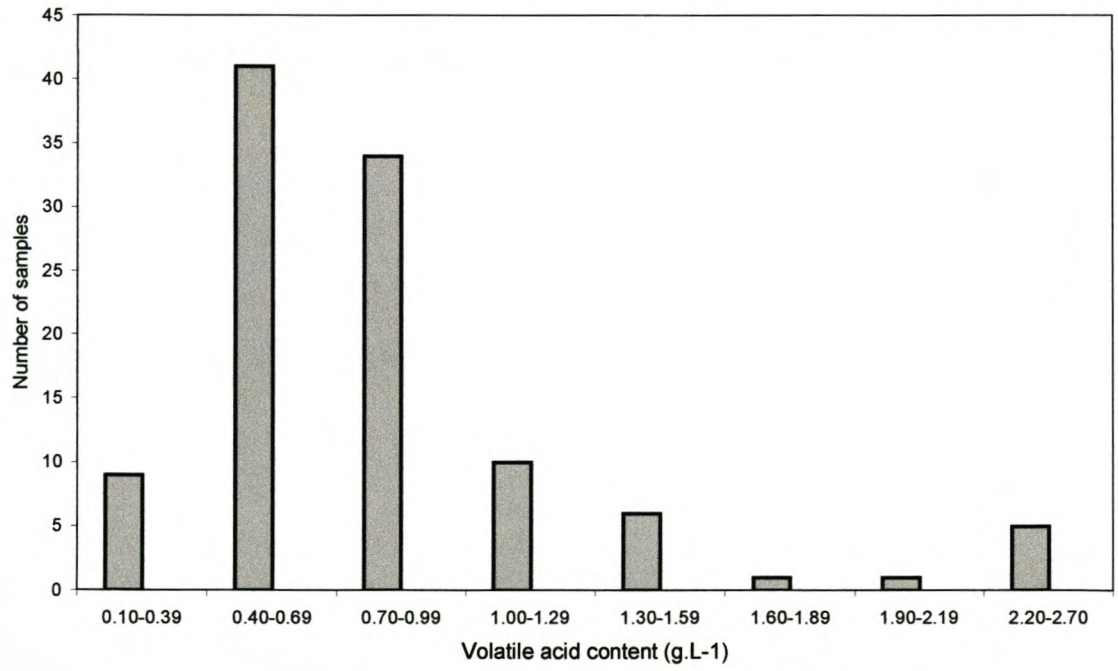

Figure 13. The distribution of samples along the volatile acid concentration range in the distilling wine sample set.

Total sulphur dioxide

One spectral outlier, an extremely turbid sample was removed from the dataset. Calibrations established for the FT-NIRS prediction of total sulphur dioxide content in distilling wine were not successful. The best regression results are summarised in Table 4. Only correlation graphs for the 1 mm measurements are shown. No correlation was found in the data between the 0.2 mm spectral measurements and the concentration data. Very poor correlations were obtained between the 1 mm spectral measurements and the total sulphur dioxide concentrations (Figure 14). The bias for the predictions was large, indicating that there was a very large systematic prediction error present in the data and great variation within the samples. As indicated in Figure 15, the predictions obtained with the two cuvettes did not differ significantly ($p \geq 0.05$). Neither of these predictions was, however, indicative of NIRS' ability to determine some organic constituents indirectly either by way of some organic moiety to which they are bound or by virtue of some effect on certain absorption bands. The distribution pattern of the total sulphur dioxide concentrations in the wine dataset (Figure 16) was also not ideal to attempt NIRS prediction on the dataset. A smaller dataset with a concentration range between 30 and 72 $mg.L^{-1}$ (shown in Table 5) produced a slight prediction improvement for the 1 mm measurements, with a higher correlation ($r = 0.39$) and lower SEP (8.7 $mg.L^{-1}$) as seen in Figure 17. The prediction for the 0.2 mm measurements deteriorated (Table 5).

The sample presentation of the wine in cuvettes was problematic, especially for a volatile constituent like sulphur dioxide. Some of the sulphur dioxide could be partially lost due to evaporation, resulting in inaccurate reference values and spectra for the samples. Gishen & Holdstock (2000) obtained a satisfactory correlation ($r = 0.83$) but a high prediction error (SEP = 23 $mg.L^{-1}$) with the Foss Winescan for total sulphur dioxide quantification in wine with a concentration range between 2 and 251 $mg.L^{-1}$. The Winescan, which operates in the mid-infrared region, was equipped with an automatic sample feeder that greatly enhanced the absorbance measurements of the wine samples and reduced loss of constituents due to evaporation.

Table 4. Summary of the independent validation statistical results obtained for total sulphur dioxide in distilling wine in the concentration range 30-120 $mg.L^{-1}$.

	0.2 mm	1 mm
Range	30 - 120	30 - 120
Mean	59	59
n_{cal}	72	72
n_{val}	34	34
r	-0.08	0.35
SEP	12.3	10.9
RMSEP	13.6	13.7
Bias	6.01	8.63
Nr. of PLS factors	1	1
RER	7.3	8.3

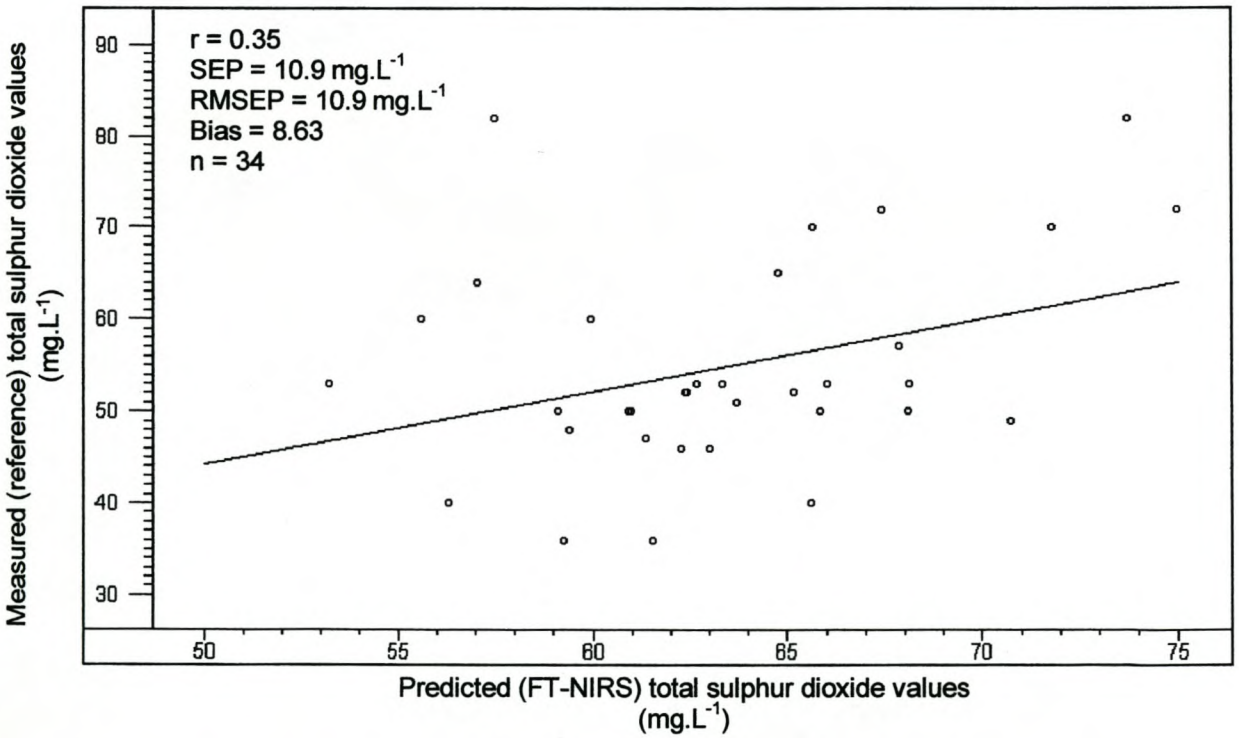

Figure 14. Plot of the predicted versus the measured total sulphur dioxide ($mg.L^{-1}$) for the distilling wine samples measured in a 1 mm cuvette in the range 30-120 $mg.L^{-1}$.

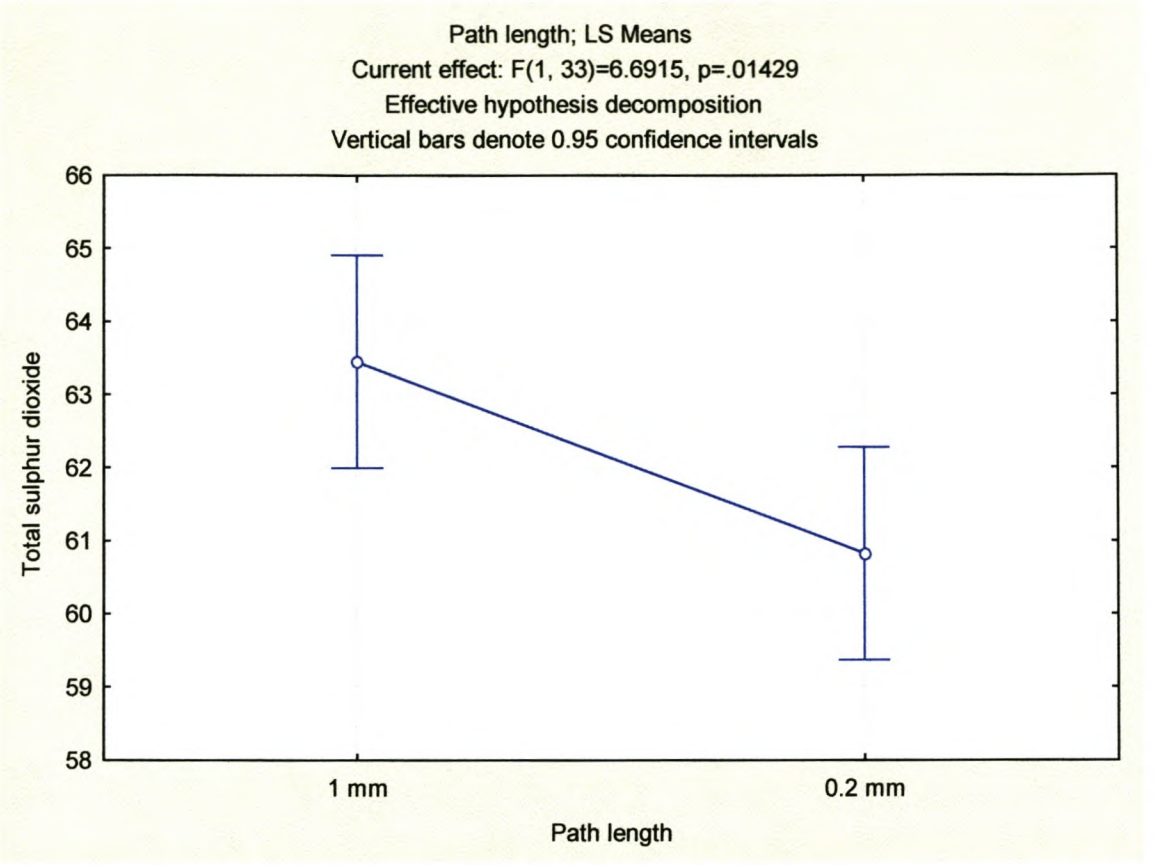

Figure 15. The ANOVA plot of the predicted values for the total sulphur dioxide content ($mg.L^{-1}$) in distilling wine for measurements in a 1 mm and a 0.2 mm cuvette.

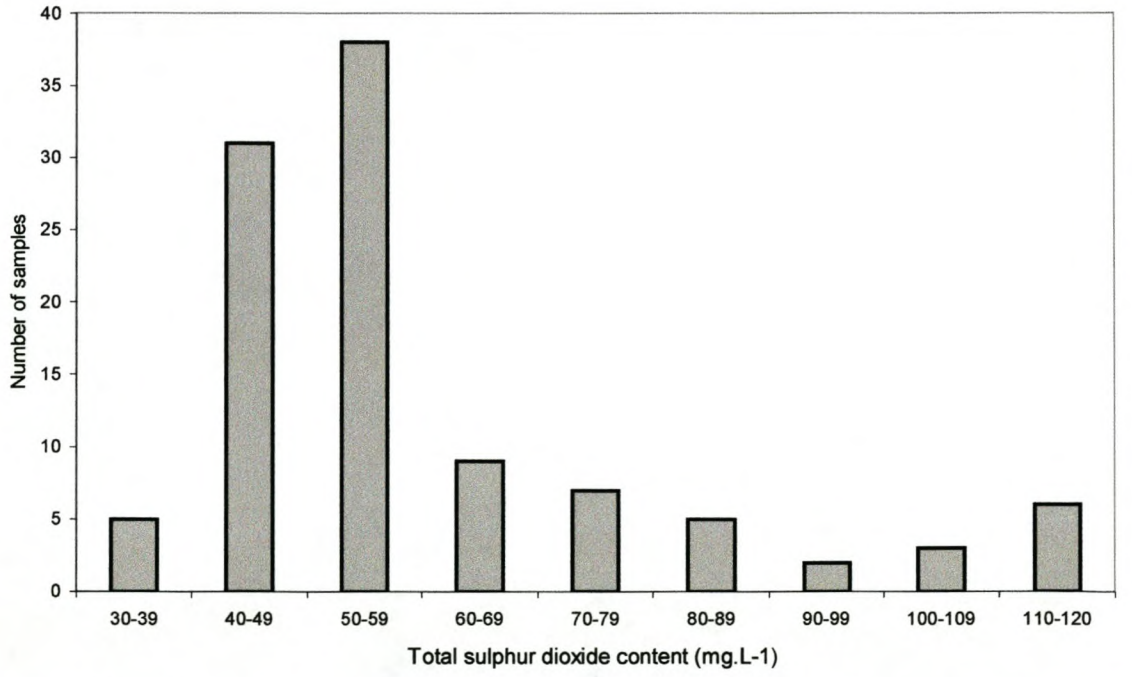

Figure 16. The distribution of samples along the total sulphur dioxide concentration range in the distilling wine samples set.

Table 5. Summary of the independent validation statistical results obtained for total sulphur dioxide in distilling wine in the concentration range 30-72 $mg.L^{-1}$.

	0.2 mm	1 mm
Range	30 – 72	30 - 72
Mean	52	52
n_{cal}	59	59
n_{val}	32	32
r	-0.25	0.39
SEP	10.1	8.7
RMSEP	10.1	8.9
Bias	-1.87	-0.05
Nr. of PLS factors	1	1
RER	4.2	4.8

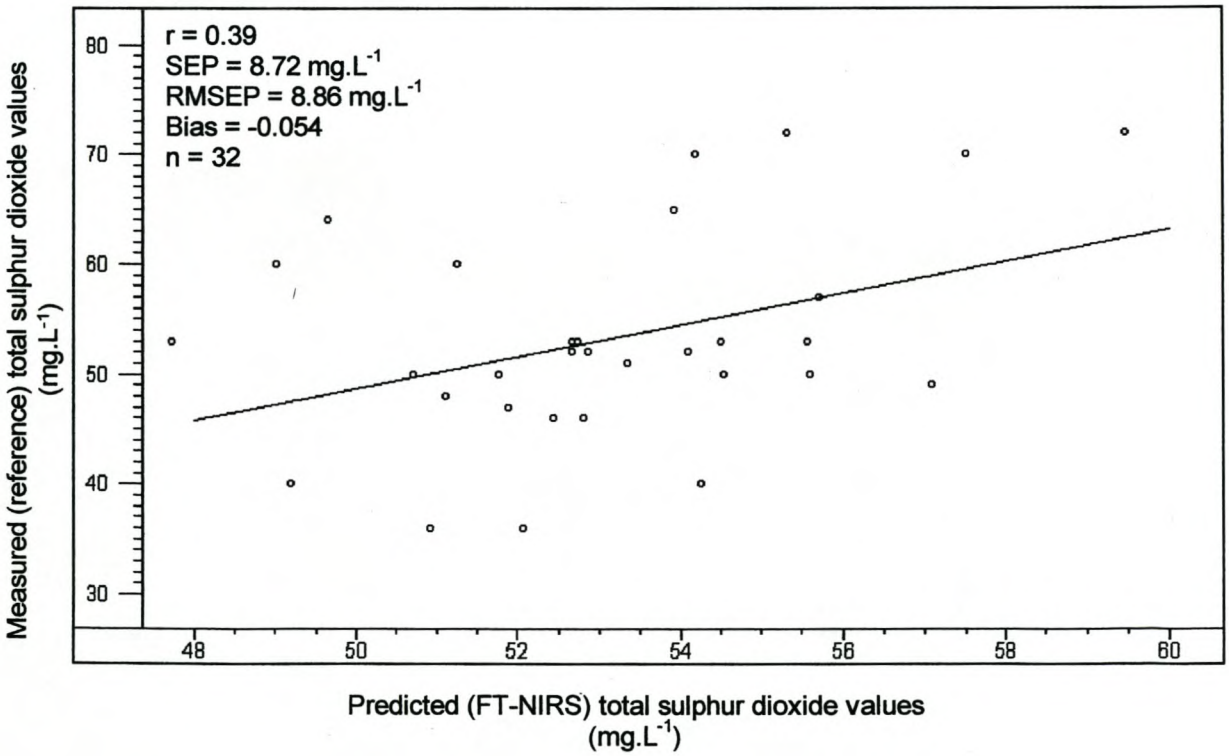

Figure 17. Plot of the predicted versus the measured total sulphur dioxide ($mg.L^{-1}$) for the distilling wine samples measured in a 1 mm cuvette in the range 30-72 $mg.L^{-1}$.

Conclusions

The evaluation of the applicability of FT-NIRS as a screening measurement for the most important quality parameters in distilling wine, confirmed the strong predictive abilities of NIRS for alcohol. It can be concluded that due to its strong absorption qualities in the near infrared region, path length does not play a significant role in alcohol measurements with NIRS. The prediction results obtained for volatile acid and total sulphur dioxide contents were unsatisfactory.

The presence of impurities in the distilling wine samples may have had a detrimental effect on the prediction performance of NIRS for the volatile acid determinations. This may have adversely affected the reference method precision and/or the near infrared spectroscopic measurements. Furthermore, sample presentation of liquids in near infrared absorbance measurements can be problematic. By using an automated liquid sampler, measurement errors, light scattering and losses due to evaporation during the measurements could be reduced. Highly volatile constituents like volatile acid and sulphur dioxide could be partially lost during sample presentation in a cell or cuvette, resulting in inaccurate reference and spectral measurements. The study also concluded that the 1 mm path length seemed the best choice for FT-NIRS quantitative analysis of constituents in wine.

The objective of this study was to develop an FT-NIRS method that could be applied for wine analysis without any pre-treatment of the samples. Due to the turbidity of the distilling wine, filtering or centrifugation of the samples before taking the NIRS measurements, might be necessary to decrease light scattering from the particles in the wine. More samples and a wider calibration range are necessary to prove the validity of the results.

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CHAPTER 4

QUANTIFICATION OF THE QUALITY PARAMETERS OF BRANDY BASE (REBATE) WINE BY NEAR INFRARED SPECTROSCOPY

CHAPTER 4

DETERMINATION OF THE QUALITY PARAMETERS OF BRANDY BASE (REBATE) WINE BY NEAR INFRARED SPECTROSCOPY

Summary

Fourier transform near infrared spectroscopy (FT-NIRS) can be used as a rapid method to measure the content of alcohol, total acid, volatile acid and total phenols as well as the pH of brandy base wine samples. PLS calibration models were derived for each of these parameters using The Unscrambler (ver. 6.11). The full cross validation results showed a strong prediction ability for the alcohol content (r = 0.92, SECV = 0.18% v/v, RER = 11.1) in brandy base wine. Good correlations and prediction statistics were obtained for the total acid contents (r = 0.89, SECV = 0.38 $g.L^{-1}$), volatile acidity (r = 0.85, SECV = 0.04 $g.L^{-1}$), total phenol content (r = 0.71, SECV = 16.4 $mg.L^{-1}$ GAE) and the pH measurements (r = 0.84, SECV = 0.09) of brandy base wine. The accuracy of these predictions as indicated by the RER values were, however, all below 10 and suggested the improvement of the reference method accuracy before attempting further calibration modelling. The prediction results obtained for the contents of acetaldehyde (r = 0.39, SECV = 1.45 $mg.L^{-1}$, RER = 3.9) and reducing sugar (r = 0.58, SECV = 0.49 $g.L^{-1}$, RER = 5.8) were unsatisfactory. The concentration range of the total sulphur dioxide dataset (18-20 $mg.L^{-1}$) was too limited to attempt NIRS modelling and prediction testing. Further investigation of FT-NIRS prediction testing with improved reference method accuracy, sample preparation and a larger sample set are suggested to improve the accuracy and therefore the validity of the results.

Introduction

Brandy base or rebate wine is produced from free run juice obtained from specific non-muscat grape cultivars including Colombar, Chenin Blanc, Ugni Blanc, Cinsaut and Palamino (Anonymous, 1988; Weitz, 2001). Skin contact is minimised and the pips and stalks prevented from being crushed into the juice, as the tannins in these components impart a bitter taste in the distilled product. Selected yeast strains are

inoculated into the juice and the fermentation regulated at 15 to 18°C. Sulphur dioxide may not be added to the wine at any stage and the lees may only be removed through centrifugation. Fermentation is terminated when the reducing sugar content is exhausted, usually after about 7 to 14 days (Weitz, 2001).

The parameters regulated in brandy base wine are important for a variety of reasons. Ethanol, the most important product of the distillation process, determines the body of any wine and affects its flavour (Burns, 1994). It serves as a basis of payment for distilling wine and is defined by legal limits in various types of alcoholic beverages. The ethanol content must be displayed on the labels of all products (Anonymous, 1996). The total acid content of wine is of importance regarding its flavour and its indirect influence on pH, colour, stability and shelf life of the product (Zoecklein *et al.*, 1994). The hydrogen ion concentration (pH) of wine affects its acidic character and serves as an indicator of defects during wine processing (Zoecklein *et al.*, 1994). Volatile acidity (almost exclusively acetic acid) is determined as a measure of microbial spoilage and plays an important organoleptic role (Burns, 1994; Anonymous, 1996). The levels of volatile acid in brandy base wine are regulated through the Liquor Products Act, Act 60 of 1989.

Sulphur dioxide is widely used in the wine industry as a chemical antioxidant and inhibitor of microbial activity (Zoecklein *et al.*, 1994). The control of sulphur dioxide in wines is very important as excess can impart an undesirable taste on the nose and palate and pose potential health risks (Ribéreau-Gayon *et al.*, 2000a). Several compounds present in grape juice and wine actively bind with sulphur dioxide resulting in unwanted volatile substances. Acetaldehyde is especially reactive with sulphur dioxide and can accumulate into certain fractions during distillation (Zoecklein *et al.*, 1994; Anonymous, 1996; Steger, 2001). The levels in brandy base wine are regulated through the South African Liquor Products Act, Act 60 of 1989.

Phenolic compounds are generally regarded as the main contributors to the colour and olfactory profile of wines and brandy (Ribéreau-Gayon *et al.*, 2000b; Zoecklein *et al.*, 1994). They are responsible for astringency and bitterness, and serve as important oxygen reservoirs. The most significant phenolic compounds found in brandy are generated through the oak maturation process, therefore lower levels of total phenols are preferred in the base wine.

Reducing sugars play multiple roles in wine processing, therefore rapid and accurate determination of the levels is important. The quality of the fermentable sugar remaining in the wine upon completion of fermentation (also called the reducing sugar content) may be important for microbial stability (Zoecklein *et al.*, 1994). In wine, these sugars can also be referred to as the residual sugars as the contents of both would be similar (Van Rensburg, 2002). Certain spoilage yeasts like *Brettanomyces/Dekkera* and lactic acid bacteria grow at sugar levels below 2 $g.L^{-1}$. The monitoring of fermentable sugars in distilling material (i.e. distilling wine and brandy base wine) is of concern in overall plant efficiency (Zoecklein *et al.*, 1994). The levels of reducing sugars in brandy base wine are regulated through the South African Liquor Products Act, Act no. 60 of 1989.

Acetaldehyde is the principle aldehyde present in wine (Zoecklein *et al.*, 1994). As an intermediate in the microbial formation of acetic acid, acetaldehyde can disrupt the vapour/liquid equilibrium at the start of distillation (Steger, 2001). This necessitates the employment of a first fraction (heads) cut-off to remove all the undesirable volatile compounds. Abnormally large amounts of acetaldehyde can, however, still accumulate into the second fraction (heart), producing unacceptable odours in the distilled product.

Screening methods are often used in routine laboratories to divide samples within a limited range from those that fall outside the permitted limits (Prichard *et al.*, 1995). Screening methods must be extremely rapid, thus permitting a high throughput of samples at low cost (Prichard *et al.*, 1995). These methods can be qualitative or semi-quantitative and may be validated only to the extent of the limit of detection by the operational laboratory. Limit of detection of a method is an indication of the minimum concentration at which a constituent can be measured by a method. Ideally, the limit of detection of the method selected should be at least one-tenth of the concentration to be measured (Prichard *et al.*, 1995).

Near infrared spectroscopy (NIRS) is an instrumental analytical technique, suitable for the rapid and reproducible measurement of the chemical composition of organic samples, requiring little or no sample preparation (Norris, 1989). The absorption of OH bonds is strong in the near infrared region, therefore the determination of alcohol and phenols is expected to work (Wetzel, 1998). The measurement of ethanol in wine and alcoholic beverages with NIRS has been well established (Kaffka & Norris, 1976; Baumgarten, 1987; Sneyd *et al.*, 1989; Garcia-

Jares & Médina, 1997; Van den Berg *et al.*, 1997). The OH first overtone band in alcohols and phenols occurs in the 1405-1425 nm region with the second overtone between 945 and 985 nm (Osborne *et al.*, 1993). Burns (1994) proposed the employment of NIRS as a future analytical technique for the determination of the total phenolic levels in wines. Esler *et al.* (2002) successfully developed rapid prediction models for the total anthocyanins concentration in red wine grapes.

Overtone bands of the carboxyl group found in carboxylic acids (organic acids) have been observed in the region of 1900 nm (Osborne *et al.*, 1993). Kaffka & Norris (1976) determined the tartaric acid content in wine using selected interference filters. The total and volatile acid levels and the pH in wine, have been predicted successfully with the Foss Winescan (Foss Electric, Denmark) by Gishen & Holdstock (2000). The Foss Winescan is a Fourier transform predictive instrument operating in the mid-infrared light range and equipped with an automatic flow system. Esler *et al.* (2002) obtained a SECV of 0.08 for the pH measurements of homogenised red wine grapes representing 10 growing regions, 10 varieties and 2 seasons.

Garcia-Jares & Medina (1997) were able to successfully predict residual sugars with NIRS in botrytized-grape sweet wines with generally higher residual sugar contents (up to 120 $mg.L^{-1}$ in extreme cases) using only 19 selected wavelengths. Gishen & Dambergs (1998) reported that the application of NIRS for the determination of residual sugar (0-10 $g.L^{-1}$) has been limited due to lack of success in obtaining reliable calibrations.

Gishen & Holdstock (2000) attempted total sulphur dioxide prediction with the Foss Winescan. A strong correlation ($r = 0.83$) was obtained but the prediction error was unacceptably high (SEP = 23 $mg.L^{-1}$) compared to the accepted standard error of laboratory (5 mg. L^{-1}).

The carbon-hydrogen bond involving the carbonyl carbon atom of an aldehyde maintains a pair of characteristic fundamental vibration bands at 3546 and 3676 nm (Osborne *et al.*, 1993). A combination band has been observed in simple saturated aldehydes in the region of 2200 nm.

Currently the alcohol, volatile acid, total phenol and acetaldehyde measurements in wine are monitored using expensive and time-consuming analytical methods. Total acids, pH, total sulphur dioxide and reducing sugars are measured with more rapid chemical or automated methods, but various consumables are still

used. A single method, such as FT-NIRS, that could simultaneously measure all the quality parameters of importance in brandy base wine, would drastically improve the selection process of suitable wine samples for brandy distillation purposes. It would be ideal as a rapid screening method that requires no sample preparation.

Objective

The objective of this study was to develop FT-NIRS methods for the determination of the alcohol, total acid, volatile acid, total sulphur dioxide, reducing sugar, total phenol and acetaldehyde contents as well as the pH of brandy base wine.

Materials and methods

Wine samples

A selection of 95 brandy base wine samples, representative of the Western Cape region and produced at different times throughout the wine season of 2002, were supplied by Distell in Worcester, South Africa. The brandy base wine samples were analysed on receipt and stored at 4°C. The samples were allowed to equilibrate to room temperature prior to recording the near infrared absorbance spectra.

Chemical analyses

Alcohol

Nearly all the alcohol present in grape wine is ethanol (ethyl alcohol) with a boiling point of 78.37°C (Anonymous, 1996). The alcohol content of the wine was determined pycnometrically by measuring the specific gravity of the wine and the alcohol-water distillate as described by the AOAC, method nr. 920.57 (AOAC, 2000).

Total acidity

The AOAC titrametric procedure nr. 962.12 for total acidity was employed, which involved a standard acid-base titration with standardised sodium hydroxide (0.1 M NaOH) and phenolphthalein as indicator (AOAC, 2000).

Volatile acidity

Steam distillation of the samples as described by Gowans (1964), was followed by titration with standardised sodium hydroxide to a phenolphthalein end point and the results reported in $g.L^{-1}$ acetic acid (AOAC, 2000).

pH

The hydrogen ion concentrations of the wine samples were measured using a Mettler Toledo 320 pH meter.

Total phenols

Total phenols were determined by means of the spectrometric method using Folin-Ciocalteu reagent as described by Singleton & Rossi (1965). The absorbance at 765 nm was measured with a Spectronic® 20 Genesys™ (Spectronic Instruments, USA). The results were reported in $mg.L^{-1}$ GAE (gallic acid equivalents).

Reducing sugar

The reducing sugar content in the brandy base wine was determined using the Lane-Eynon procedure (AOAC method nr. 920.64) which involved the reduction of Cu (II) under alkaline conditions (AOAC, 2000).

Acetaldehyde

The acetaldehyde concentration in the brandy base wine samples was determined by means of the microextraction method of Ferreira *et al.* (1993), with a HP 6890 series gas chromatograph fitted with a FID, split-splitless injector and an automatic sampler 7683 on a Supelco SPB5 column. Only 74 of the 95 samples were analysed to determine their acetaldehyde concentrations.

Total sulphur dioxide

The sulphur dioxide content of the brandy base wine was determined iodometrically by potassium iodate/iodide using the AOAC (nr. 940.20) Ripper method (Anonymous, 1996; AOAC, 2000).

Fourier transform near infrared spectroscopy (FT-NIRS) measurements

Near infrared spectra were recorded in transmittance mode at 2 nm intervals using a Perkin-Elmer Spectrum Identicheck™ 2.0 FT-NIRS system (Perkin-Elmer corp., Norwalk, CT., U.S.A.). The spectra were collected between 650 and 2500 nm, with a 16 scan sequence at a resolution of 16 cm^{-1}. The liquid samples were presented in a 0.2 mm path length Quartz cuvette (Helma).

Data analysis

Spectra were exported from the Perkin-Elmer *.sp format as ASCII files and converted via a macro reader into The Unscrambler ver. 6.11 software (CAMO AS, Trondheim, Norway). Multiplicative scatter correction (MSC) was applied to the spectra to eliminate the baseline fluctuations arising from light scattering and density variations. For the acetaldehyde, pH, reducing sugar and volatile acid modelling, a Savitsky-Golay second derivative transformation with a nine-point segment was applied to remove additive and multiplicative baseline variations in the spectra. The partial least squares (PLS) algorithm was used to relate the spectral and chemical data to bilinear models. The spectral and chemical data were projected onto a limited number of latent orthogonal factors, retaining the principal information for both spectral and chemical data. Various concentration and wavelength ranges were examined to determine the optimum conditions for the modelling of each component.

The square root of the coefficient of determination, simply called the correlation coefficient (r), was used to describe the linear relationship between the chemical analysis and the NIRS analysis (equation 1).

$$r = \frac{\sum_{i=1}^{n}\left(\hat{y}_i - \bar{y}\right)^2}{\sqrt{\sum_{i=1}^{n}\left(y_i - \bar{y}\right)^2}} \qquad \text{.....1}$$

where y_i = the reference value for the i^{th} sample

$\hat{y}_i$ = the predicted value for the i^{th} sample

$\bar{y}$ = the mean *y* value for all the samples

n = the number of samples

The standard error of laboratory (SEL) is an indication of the reference method repeatability (equation 2) and can be referred to as the precision of the conventional chemical analysis (Westerhaus, 1989). The comparison between the SEL of a reference method and the SECV (standard error of cross validation) of the NIRS method was used to asses the performance of the NIRS calibration. Given that

chemical reference methods were used to develop the NIRS calibrations, the SECV could therefore not be expected to be lower than the SEL of the reference method.

$$SEL = \sqrt{\frac{\sum\left(y_1 - y_2\right)^2}{2n}} \qquad \ldots\ldots\ldots\ldots 2$$

where y_1 and y_2 = results of duplicate determinations

n = the number of samples

Full cross validation was performed to test the accuracy of the PLS models for predicting the chemical composition of brandy base wine samples. Full cross validation is regarded as a better representation of the true efficiency of a calibration equation when the sample set is small (Williams & Sobering, 1996). For full cross validation, successive samples were deleted from the calibration set, one at a time. After every deletion, a calibration was performed on the rest of the samples, before being tested on the removed sample (Martens & Næs, 1989; Næs & Isaksson, 1991).

The accuracy of the prediction testing applied to the validation set was expressed as the standard error of cross validation of the bias-corrected residual (SECV) as shown in equation 3. It was used to express the estimate of the magnitude of the error when independent samples were predicted using the models. It allowed for comparison between the NIRS predicted values and the reference values. The root mean square error of cross validation (RMSECV) incorporated the bias-effect to prevent an over-optimistic impression of the prediction error (equation 4).

$$SECV = \sqrt{\frac{\sum_{i-1}^{n}\left(y_i - \hat{y}_i - Bias\right)^2}{n-1}} \qquad \ldots\ldots\ldots\ldots 3$$

$$RMSECV = \sqrt{\frac{\sum_{i-1}^{n}\left(y_i - \hat{y}_i\right)^2}{n}} \qquad \ldots\ldots\ldots\ldots 4$$

where y_i = the reference value for the i^{th} sample

$\hat{y}_i$ = the predicted value for the i^{th} sample

n = the number of samples

Bias (equation 5) has a strong random component that can change from one validation set to another and need to be considered. The bias is interpreted as the average difference between y and $\hat{y}$ in the prediction set (Næs & Isaksson, 1992).

$$\text{Bias} = \frac{1}{n}\sum_{i-1}^{n}\left(y_i - \hat{y}_i\right) \qquad \text{.....5}$$

The prediction performance of the NIRS models as it relates to the concentration range of the chemical measurements, were evaluated lastly with the range over error ratio (RER statistic) (Williams & Sobering, 1996). The RER statistic was employed instead of the RPD statistic (standard deviation/SECV) as the range of the dataset and therefore the variance in the original reference data was limited. The RER statistic is the ratio of the range (max. value minus min. value) of the reference data for the validation samples to the SECV (Range/SECV). The RER value should ideally be at least 10, indicating that the equation is capable of predicting the required values with an accuracy of at least one tenth of the range (Williams & Sobering, 1996). According to Prichard *et* al. (1995), the limit of detection of a screening method should be at least one-tenth of the concentration to be measured.

Results and discussion

The near infrared spectra of organic products tend to be dominated by the water present in the samples. Quantitative analysis therefore relies on minor changes in the spectra. Absorption spectra, that closely resemble a water spectrum, as shown in Figure 1, were obtained for a 10% ethanol solution and two spectrally different brandy base wine samples. The first overtone water bands can be seen at 1934 nm and 1454 nm while the second overtone water bands are visible at 967 nm and 715 nm. This is the most well-known of all absorptions in the near infrared region and is due to the combination of OH stretch and OH bend vibrations of the water molecule. A peak is visible at 2265 nm in the spectra of the aqueous-alcohol solution

and two wine samples. This is a combination band of CH stretch and CH bend vibrations of the ethanol molecule.

One outlier was removed during the regression of the parameters after a preliminary study revealed its outlying status. The outlier especially influenced the total acid and pH prediction abilities. Principal component analysis (Figure 2) confirmed this sample to be an outlier. The sample differed spectrally from the other samples in the set but the reference data did not reveal any abnormalities. This was probably due to interference during the spectral measurement. The calibrations were developed with brandy base wine samples obtained from a commercial distillery. The concentration ranges of most of the components in the wine were therefore limited, but they did cover the concentration ranges that could be expected in typical brandy base wine samples in future. The best calibration results were obtained over the entire wavelength range (650-2500 nm) and covering the entire concentration range of each constituents' dataset.

Alcohol

The results of the full cross validation statistics for alcohol prediction testing in brandy base wine are shown in Table 1 and Figure 3. A high correlation coefficient was obtained for the FT-NIRS prediction of alcohol in the brandy base wine samples ($r = 0.92$). The standard error of cross validation (0.18 v/v) when using 6 PLS factors, was high relative to the standard error of laboratory of 0.1% v/v. It was also higher than the SEP obtained during previous studies of alcohol predictions by Gishen & Holdstock (2000) with the Foss Winescan FT 120 (SEP = 0.075% v/v) and Garcia-Jares & Mèdina (1997) using 19 selected interference filters (SEP = 0.065% v/v).

The sampling procedure of the liquid samples in 0.2 mm path length cuvettes could have played an important role in the lower accuracy and precision obtained for the results in this study. Sample saturation best be avoided during NIRS absorbance measurements, but cuvettes with a longer path length (0.5 mm) or automatic sampling would probably be a better option for wine absorption measurements with FT-NIRS.

The RER values of 11.1 indicated that the accuracy of the equations were acceptable and the SECV of the models reported in this work are sufficiently low to

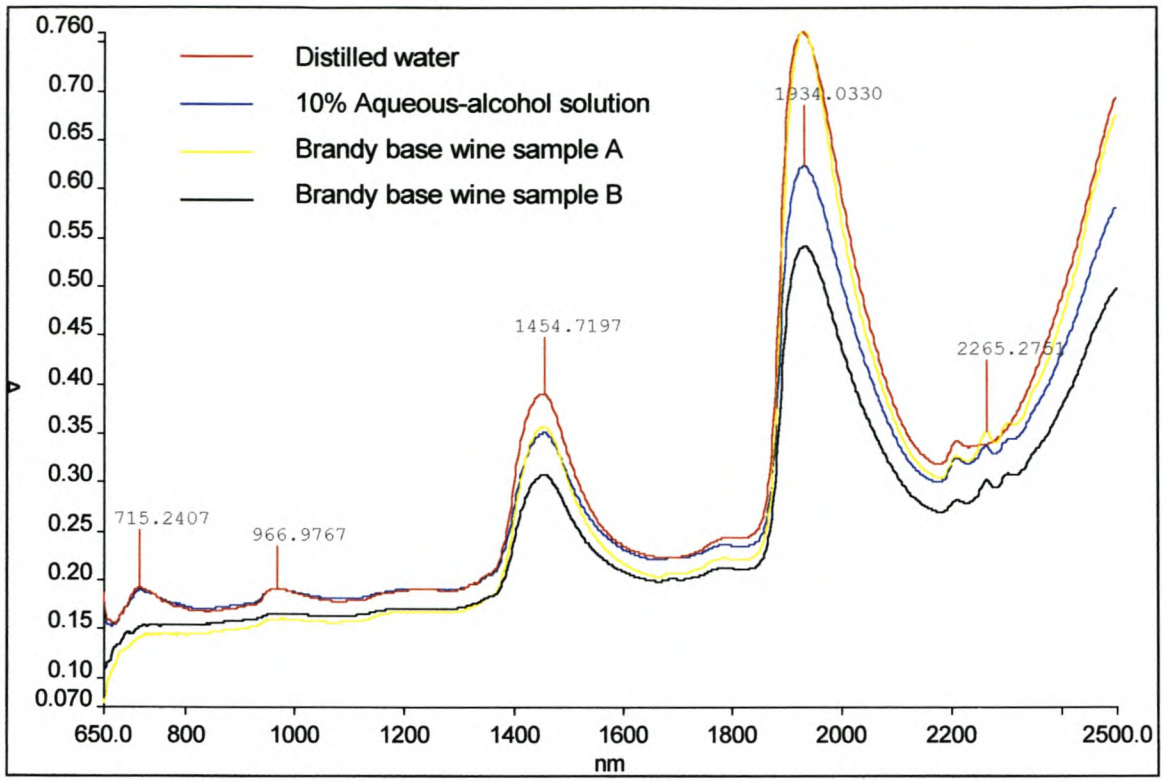

Figure 1. Absorption spectra of distilled water, a 10% aqueous-ethanol solution and two brandy base wine samples showing the main absorption peaks.

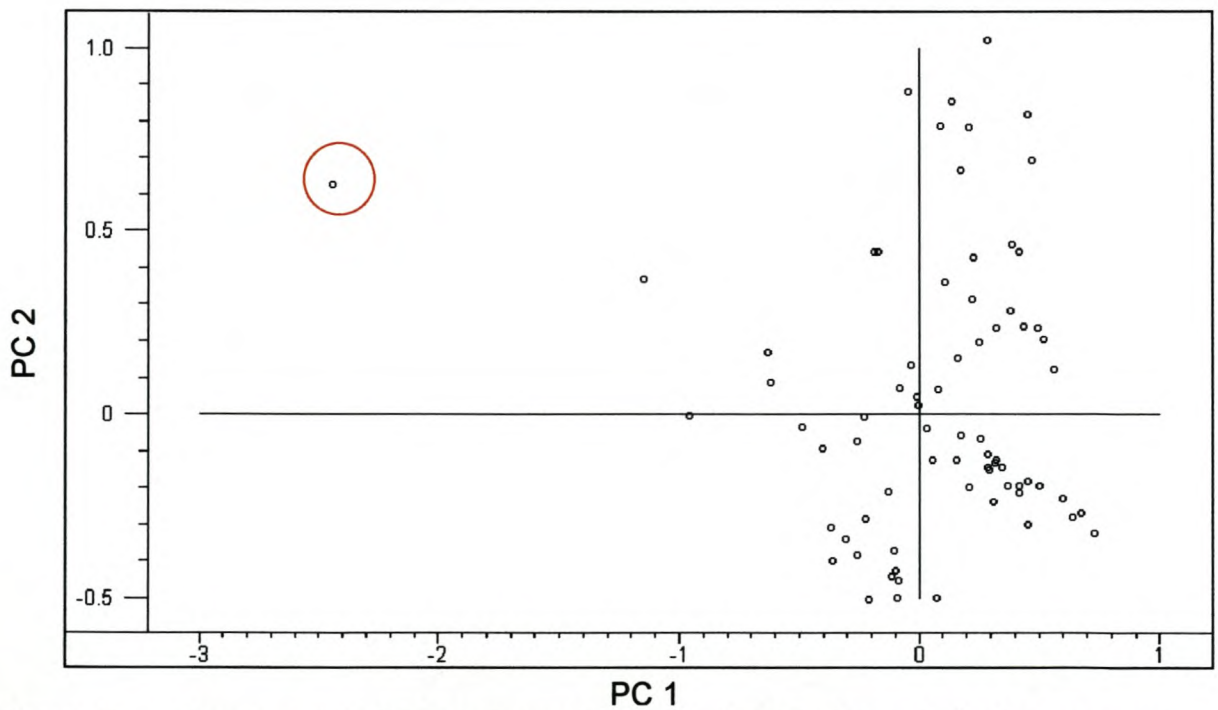

Figure 2. A score plot of PC 1 versus PC 2 obtained during principal component analysis on the raw spectral data to indicate the outlier (circled) in the dataset.

recommend FT-NIRS as a screening method for alcohol in brandy base wine. The plots of the residual variance of the concentration data against the PLS factors (Figure 4) showed that 6 PLS factors were sufficient to develop a robust calibration, without overfitting. Excellent prediction statistics were obtained when 8 (r= 0.97; SECV = 0.11% v/v) and more PLS factors were employed, but at the risk of decreasing the robustness of the calibration. The strong correlation for alcohol was expected, given that the measurement of alcohol in wine and alcoholic beverages has been well established (Kaffka & Norris, 1976; Baumgarten, 1987; Sneyd *et al.*, 1989; Garcia-Jares & Médina, 1997; Van den Berg *et al.*, 1997).

Table 1. Summary of full cross validation prediction results for the ethanol contents, total acidity, volatile acidity and pH of brandy base wine samples.

	Ethanol % v/v	**Total acid $g.L^{-1}$**	**Volatile acid $g.L^{-1}$**	**pH**
Range	10.9-12.9	3.6-6.9	0.2-0.5	3.3-4.0
Mean	12.1	5.0	0.3	3.7
r	0.92	0.89	0.85	0.84
SECV	0.18	0.38	0.04	0.09
RMSECV	0.18	0.38	0.04	0.09
Bias	-0.002	-0.003	-0.001	0.0028
Nr. of PLS factors	6	5	9	3
RER	11.1	8.7	4.3	7.8

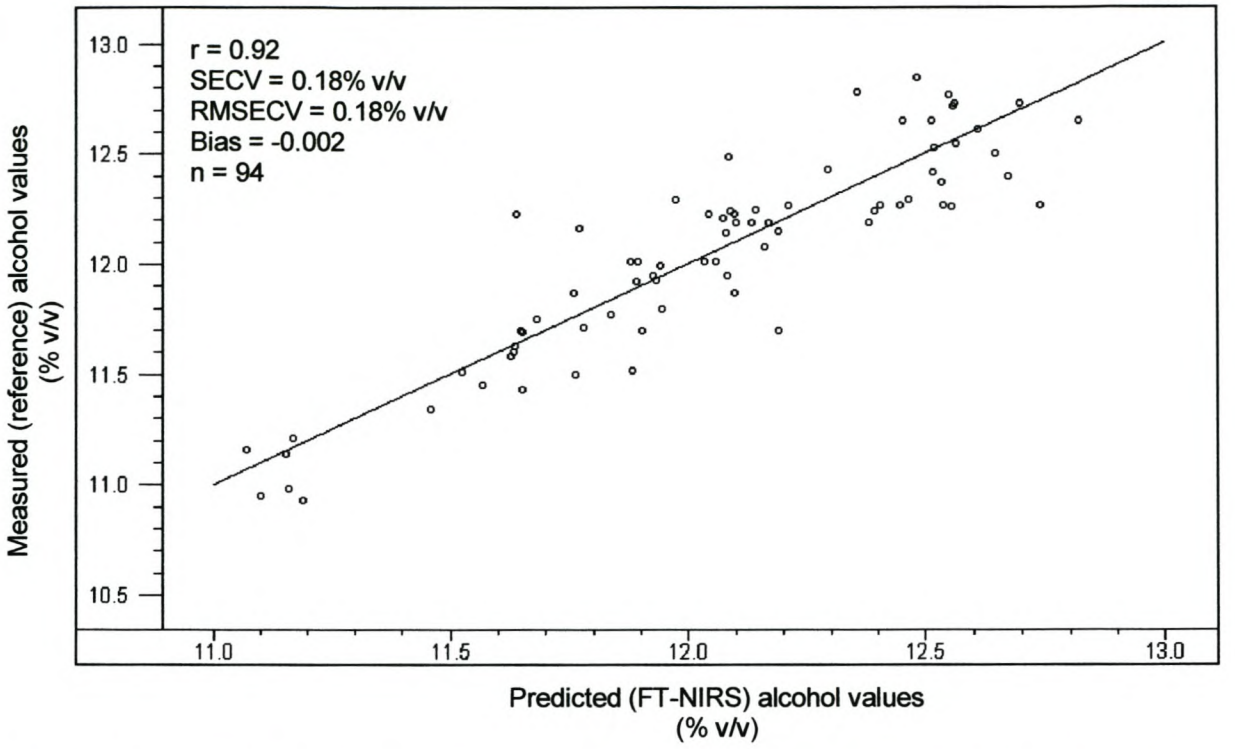

Figure 3. Predicted versus measured ethanol concentration (% v/v) of the full cross-validated brandy base wine samples.

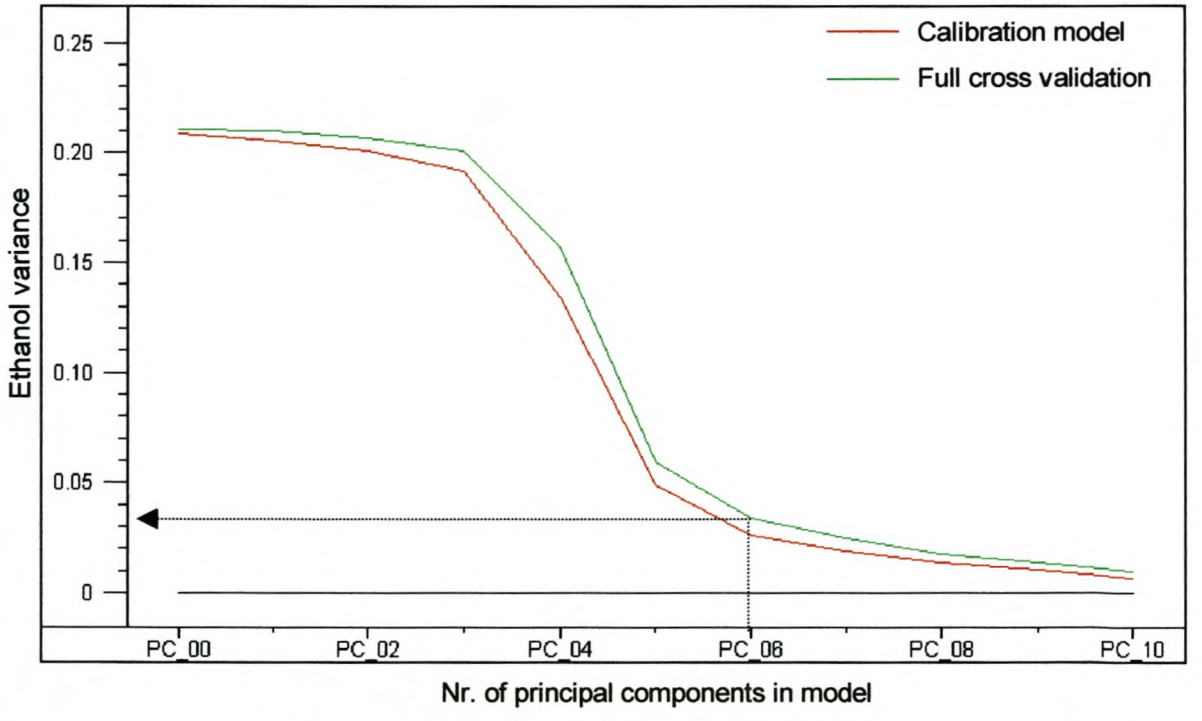

Figure 4. The residual variance plot obtained for the ethanol calibration and full cross validation models of brandy base wine.

Total acid

Prediction of total acid yielded satisfactory results and a strong correlation (r = 0.89) existed for the full cross-validated data set (Table 1 & Figure 5). The plot of the residual variance of the concentration data against the PLS factors in Figure 6, indicated that no overfitting occurred during the modelling of total acid using 5 PLS factors. Adding more factors to the calibration models would probably have resulted in overfitting and decreased robustness. The ideal would have been to follow the local minimum approach, whereby only 2 PLS factors were modelled. In this case the SECV would have doubled, resulting in a very robust, but rather inaccurate model. This model can therefore not be presented as an immediate solution to total acid prediction in brandy base wine.

Compared to the SEL of the reference method (0.2 $g.L^{-1}$) and the SEP obtained for total acid during a study by Gishen & Holdstock (2000) with the Foss Winescan (SEP = 0.17 $g.L^{-1}$), the prediction error (SECV = 0.38 $g.L^{-1}$) obtained for the model was high. The RMSECV had the same value as the SECV due to the small bias effect. The RER value showed poor accuracy (8.7) and caution should, therefore, be applied until further investigation with a larger sample set and more sensitive reference values (obtained with HPLC or GC) can confirm the suitability of FT-NIRS for robust total acid measurements in brandy base wine with acceptable accuracy.

Volatile acid

The correlation for the volatile acid concentration in brandy base wine by FT-NIRS yielded satisfactory results. The best equation for the prediction of volatile acidity in brandy base wine samples had a correlation coefficient of 0.85 and a SECV of 0.04 $g.L^{-1}$ (Table 1 & Figure 7). This compared extremely well with the accepted standard error of laboratory of 0.05 $g.L^{-1}$ and to the prediction results (r = 0.90, SEP = 0.06 $g.L^{-1}$) obtained in a study on the Foss Winescan (Gishen & Holdstock, 2000). The RMSECV had the same value as the SECV due to the small bias effect. The plot of the residual variance of the concentration data against the number of PLS factors (9) used in the modelling in Figure 8 showed that there was no overfitting of data during the modelling. The RER value of 4.3 obtained for the prediction, however, showed very poor accuracy of the prediction. The concentration range of

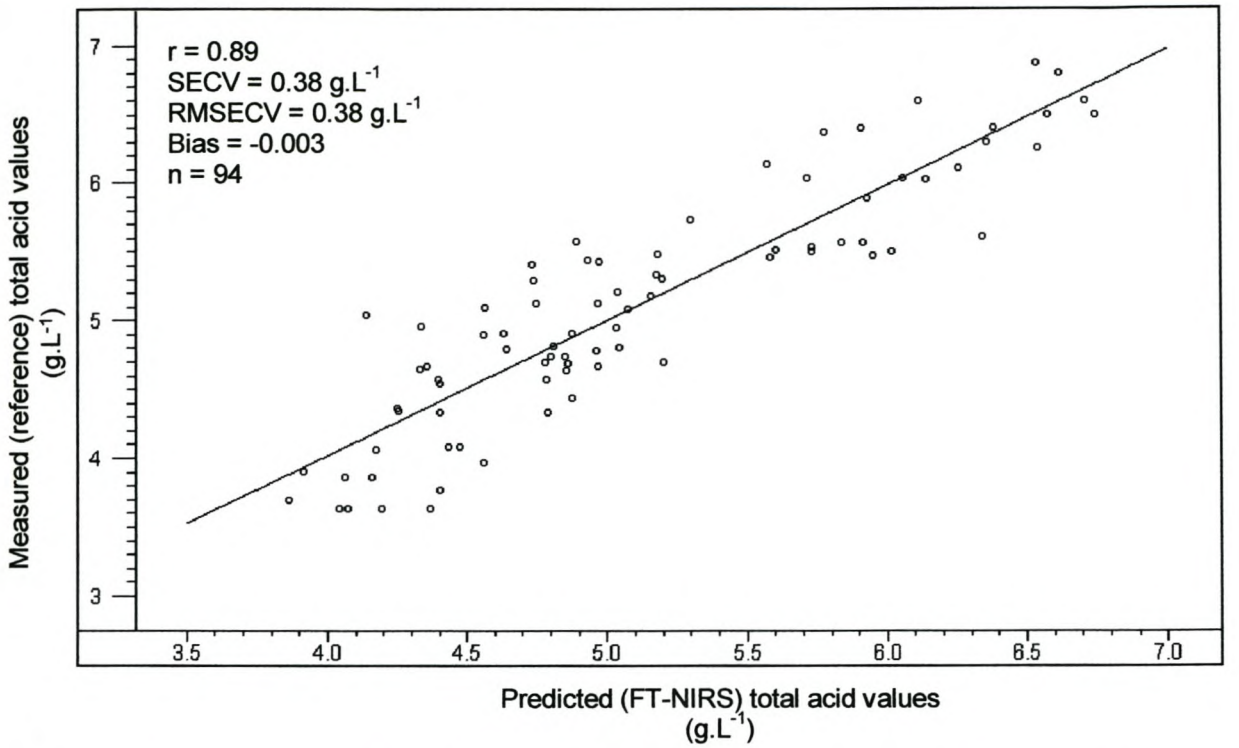

Figure 5. Predicted versus measured total acid content ($g.L^{-1}$) for the full cross-validated brandy base wine samples.

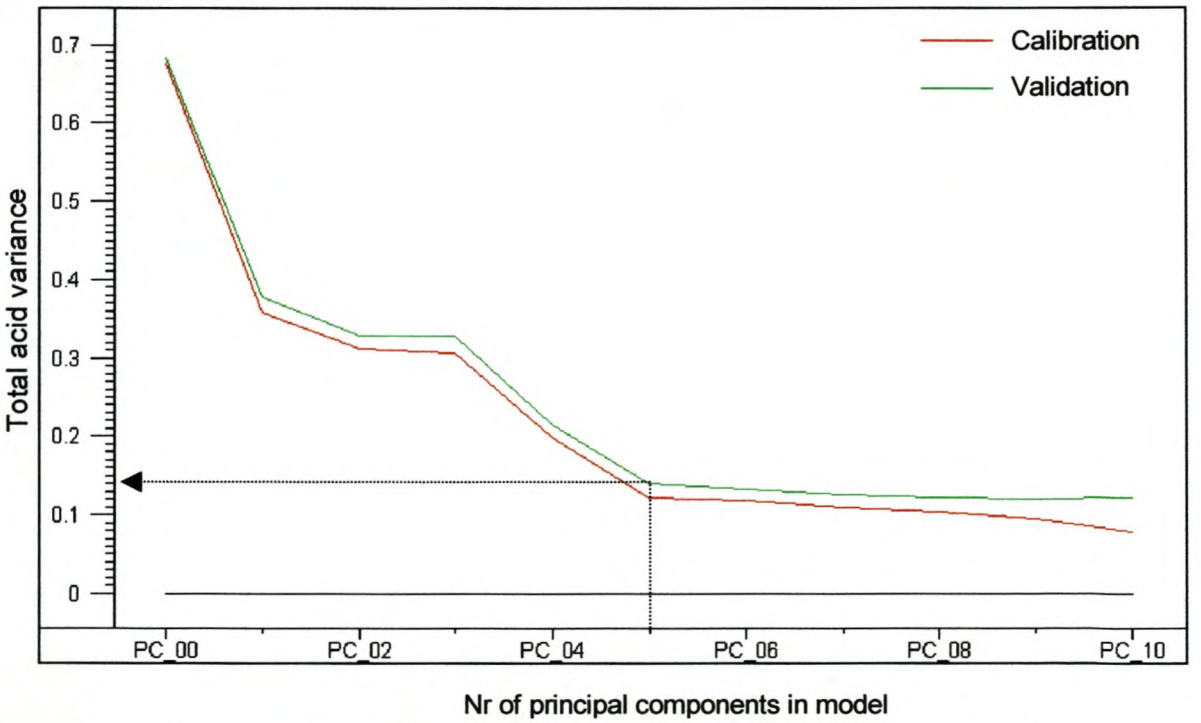

Figure 6. The residual variance plot obtained for the total acid calibration and full cross validation models of brandy base wine.

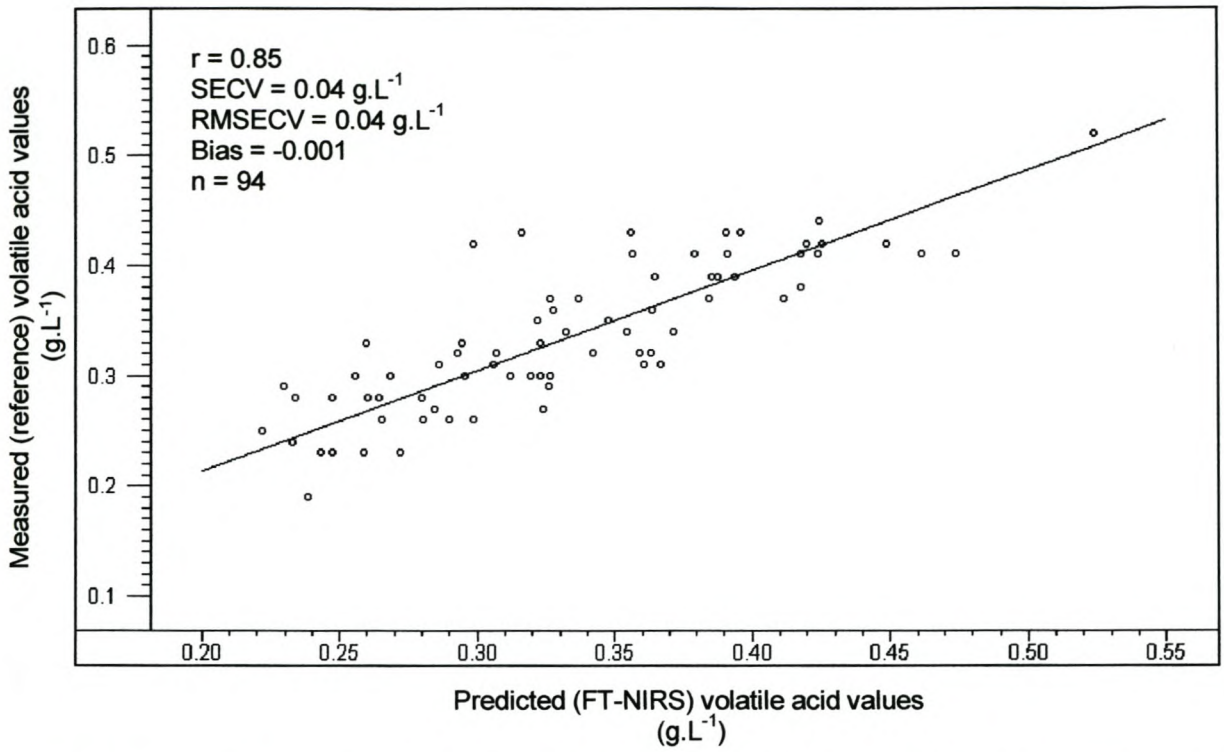

Figure 7. Predicted versus measured volatile acidity ($g.L^{-1}$) for the full cross-validated brandy base wine samples.

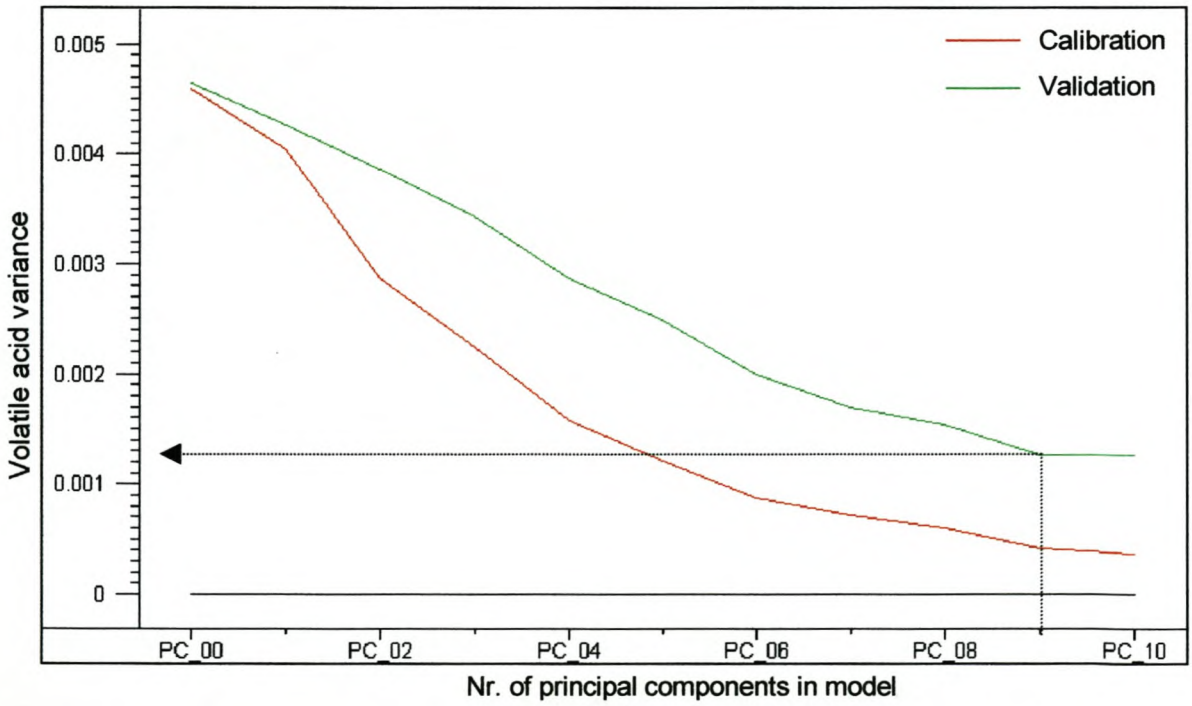

Figure 8. The residual variance plot obtained for the volatile acid calibration and full cross validation models of brandy base wine.

the volatile acid values were very narrow, indicating that caution should be applied before applying the method for volatile acid measurements in brandy base wine. Further investigation into the volatile acidity prediction abilities of FT-NIRS must be made by incorporating more samples and a considerable wider concentration range into the calibration equation and employing a more sensitive reference method such as HPLC or GC.

pH

Good predictions were obtained for the pH of the wines with a correlation coefficient of 0.84 found between the FT-NIR spectral data and reference values (Table 1 & Figure 9). The SECV of 0.09 were higher than the uncertainty of the reference method (SEL = 0.05) and the SEP obtained in a study by Gishen & Holdstock on the Foss winescan (0.06). Esler *et al.*, (2002), however, obtained a SECV of 0.08 for the pH measurements of homogenised red wine grapes representing 10 growing regions, 10 varieties and 2 seasons. More PLS factors could have been used for the calibration modelling to improve the accuracy, but to ensure the robustness of the equation only 3 PLS factors were employed (Figure 10). The RER value of 7.8 was low and indicated a poor accuracy for this specific model. Only by expanding the range of the sample set, can a clear indication of the suitability of FT-NIRS for pH prediction on brandy base wine samples in future be found. It is unlikely that the NIRS prediction of pH is limited by the reference method precision.

Total phenols

A good relationship existed between the total phenol content and FT-NIR spectroscopic data. The full cross validation produced a highest correlation coefficient of 0.71 as shown in Table 2 and Figure 11. The SECV of 16.4 $mg.L^{-1}$ GAE were high compared to the uncertainty of the reference method (10 $mg.L^{-1}$ GAE). The RER value of 8.2 obtained for the full cross validation of the 7 PLS factor model indicated that the accuracy of the equations were too low for future accurate prediction measurements of total phenols in brandy base wine. The plot of the residual variance of the concentration data against the PLS factors (Figure 12) showed that no overfitting occurred during the modelling of the components to the spectral data. Improvement of the reference method accuracy could have a

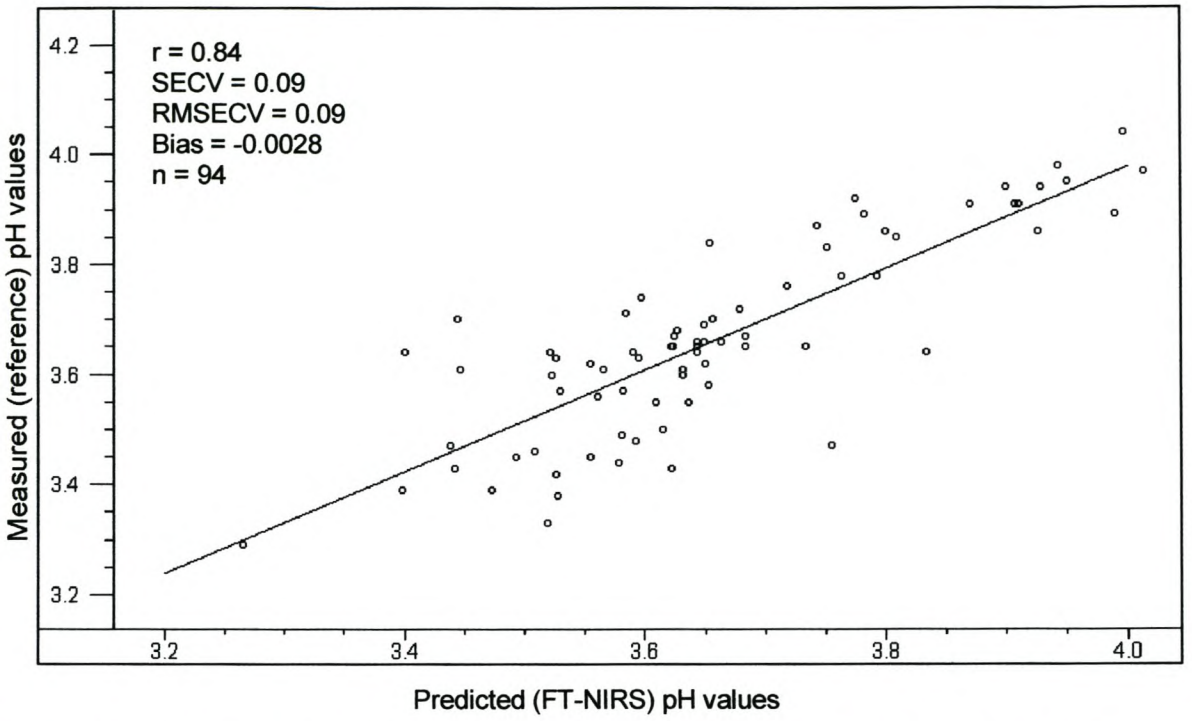

Figure 9. Predicted versus measured pH values for the full cross-validated brandy base wine samples.

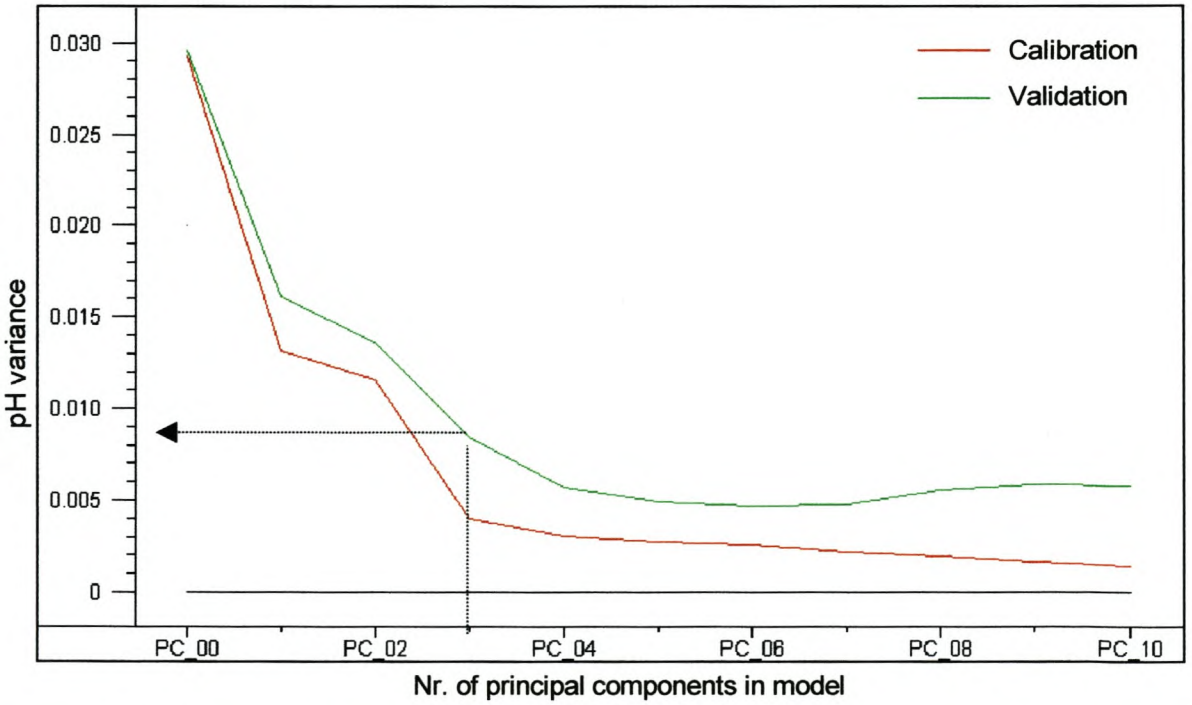

Figure 10. The residual variance plot obtained for the pH calibration and full cross validation models of brandy base wine.

significant effect on the FT-NIRS prediction accuracy of total phenols in wine as the OH-bands of phenolic groups have strong absorption peaks in the near infrared region. More selective and specific analytical methods such as HPLC and GC could aid in the FT-NIRS prediction measurements of total phenols in wine in future. Lower levels of total phenols are expected in the brandy base wine, therefore, the spectral measurement of the wine in a 1 mm path length cuvette instead of a 0.2 mm path length cuvette is suggested for future applications.

Table 2. Summary of full cross validation prediction results obtained for the total phenol, reducing sugar and acetaldehyde contents of brandy base wine samples.

	Total phenols $mg.L^{-1}$ GAE	**Reducing sugar $mg.L^{-1}$**	**Acetaldehyde $mg.L^{-1}$**
Range	102-236	0.8-3.6	5.6-11.2
Mean	183.9	2.0	7.7
r	0.71	0.58	0.39
SECV	16.4	0.49	1.45
RMSECV	16.4	0.49	1.44
Bias	0.472	0.002	0.02
Nr. of PLS factors	7	7	2
RER	8.2	5.8	3.9

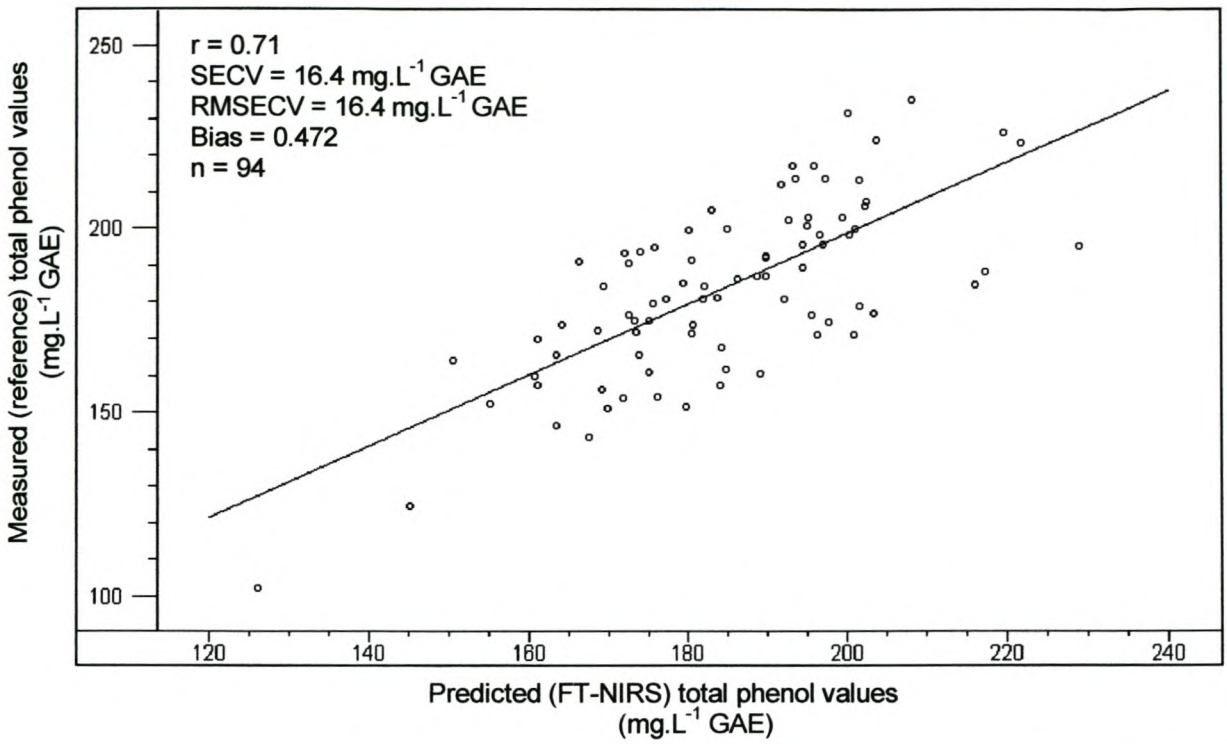

Figure 11. Predicted versus measured total phenol concentration (mg.L^{-1} GAE) of the full cross-validated brandy base wine samples.

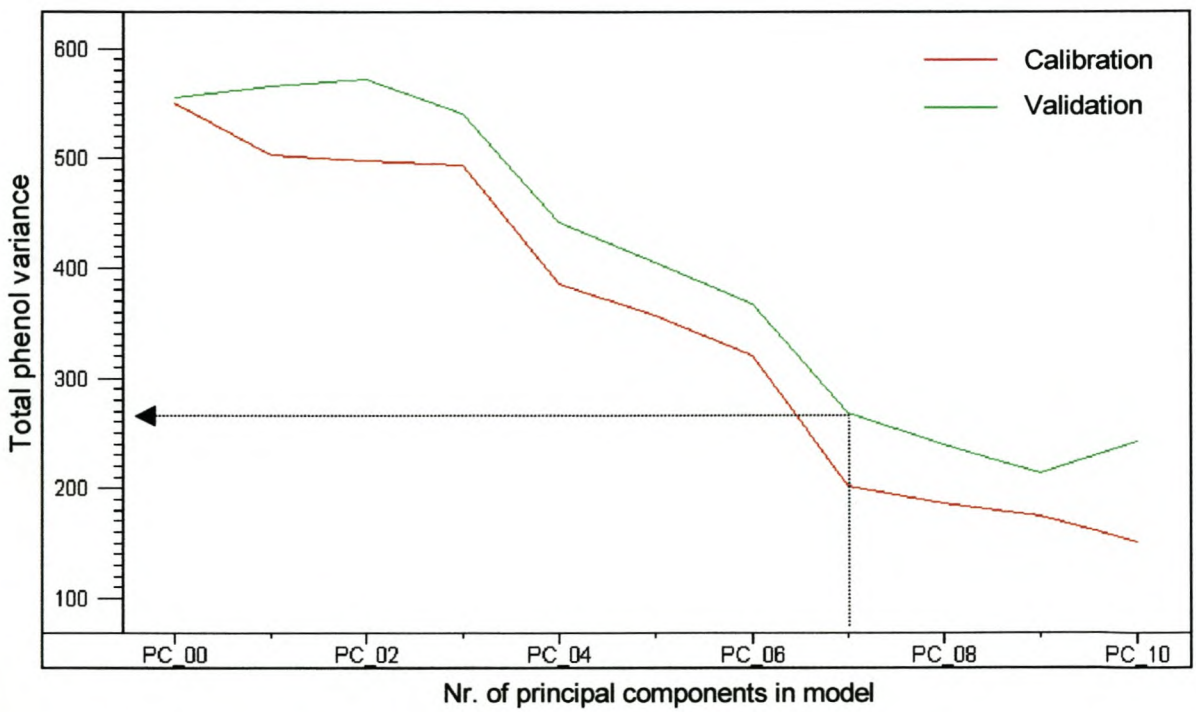

Figure 12. The residual variance plot obtained for the total phenol calibration and full cross validation models of brandy base wine.

Reducing sugar

The correlation coefficient between the reducing sugar concentration data and the near infrared spectral measurements of brandy base wine was low. The best correlation achieved for the full cross validation had a correlation coefficient of 0.58 with a high prediction error (SECV of 0.49 $g.L^{-1}$) as shown in Table 2 and Figure 13. Figure 14 shows the residual variance plot for the full cross validation set. Employing a greater number of PLS factors could not improve the modelling of the reducing sugar concentration data against the FT-NIRS data.

Absorption bands of glucose, one of the most important reducing sugars in wine, have been observed at 1480 and 1580 nm (Osborne *et al.*, 1993). The quantities present in the brandy base wine were probably too low to be of any analytical use and the path length of the cuvette was probably too short for detection purposes. Gishen & Dambergs (1998) reported that the application of NIRS for the determination of residual sugar (0-10 $g.L^{-1}$) has been limited due to lack of success in obtaining reliable calibrations.

Acetaldehyde

Only 74 brandy base wine samples were analysed in terms of their acetaldehyde concentrations. The weak correlation obtained for the full cross validation ($r = 0.39$) and the high SECV (1.45 $mg.L^{-1}$) as shown in Table 2 and Figure 15, were not acceptable for analytical purposes. The RER value of 3.9 indicated that the model; had very poor prediction abilities and low accuracy. The residual variance plot in Figure 16 showed that a greater number of PLS factors would definitely have resulted in overfitting of the data.

The carbon-hydrogen bond involving the carbonyl carbon atom of an aldehyde has a pair of characteristic fundamental vibration bands at 3546 and 3676 nm. A combination band has been observed in simple saturated aldehydes in the region of 2200 nm. The second overtone at 1960 nm in aldehydes is probably too weak and too close to the water band at 1940 nm, to be of analytical use in a complex matrix like wine. The low concentration range of the acetaldehyde in the wine samples, could also have contributed to the poor calibration results, as these were probably below the detection limit of the equipments' detectors.

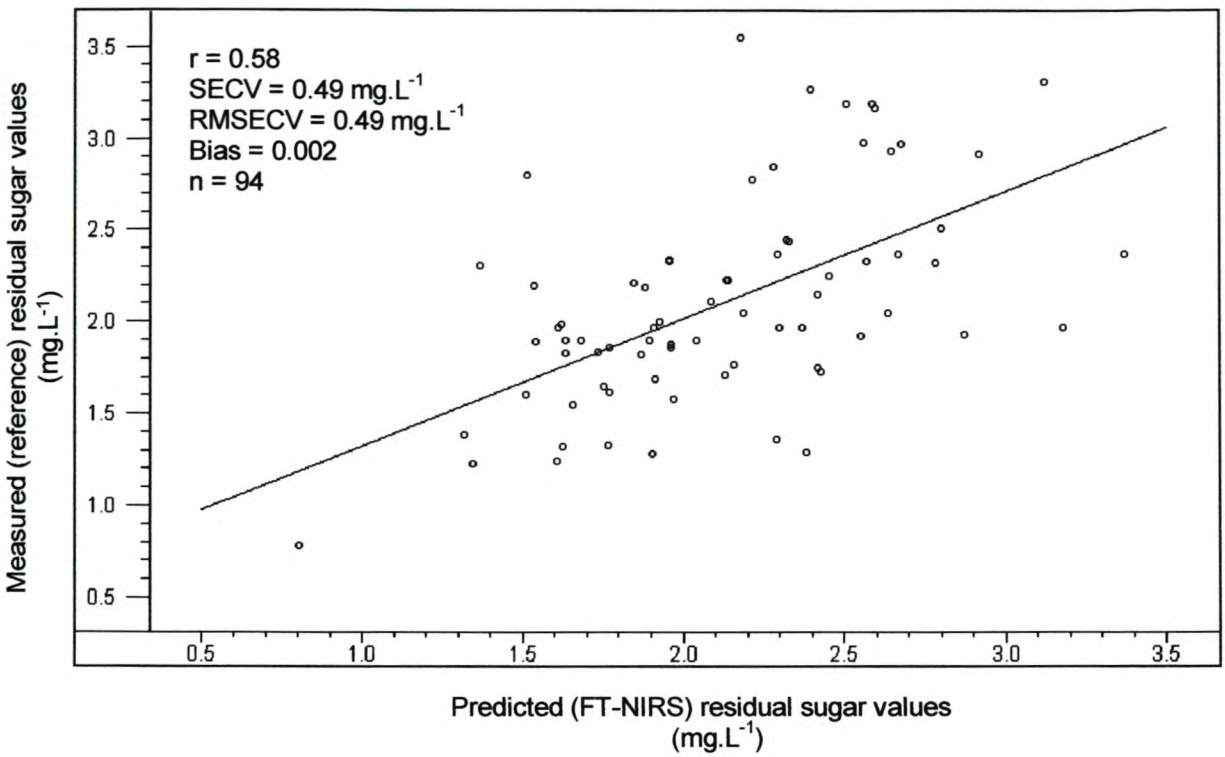

Figure 13. Predicted versus measured reducing sugar concentrations ($mg.L^{-1}$) for the full cross-validated brandy base wine samples.

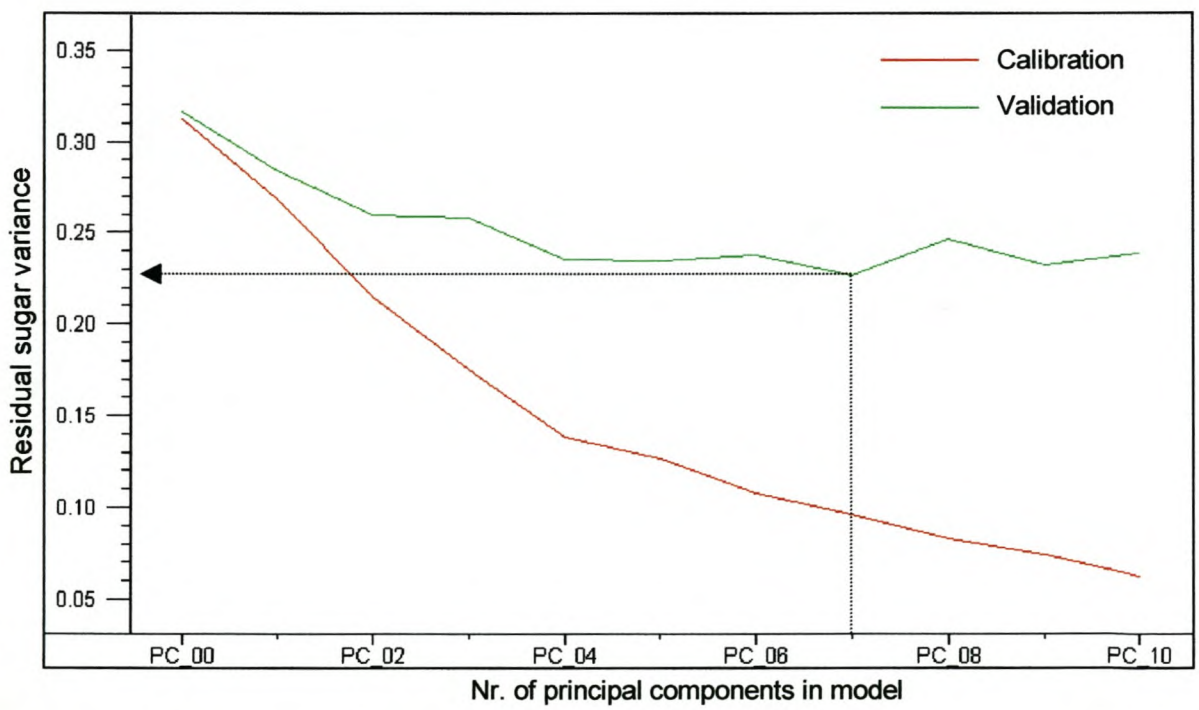

Figure 14. The residual variance plot obtained for the reducing sugar calibration and full cross validation models of brandy base wine.

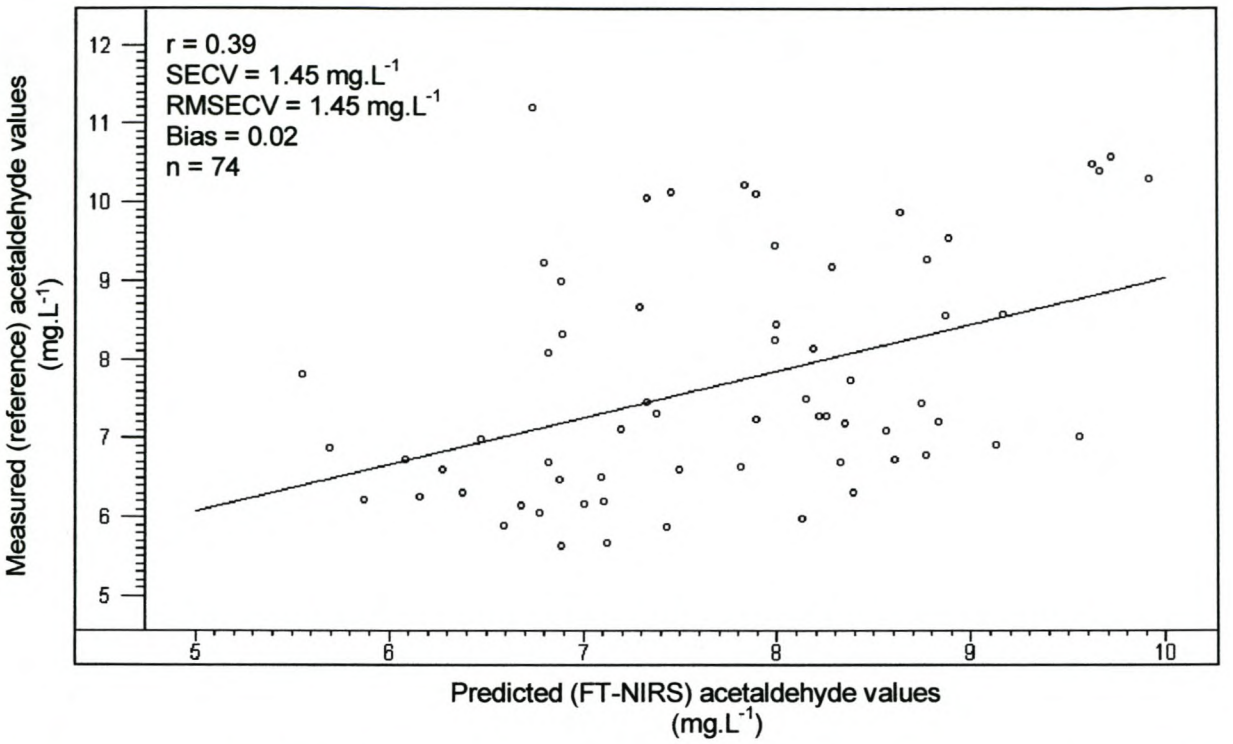

Figure 15. Predicted versus measured acetaldehyde concentrations ($mg.L^{-1}$) for the full cross-validated brandy base wine samples.

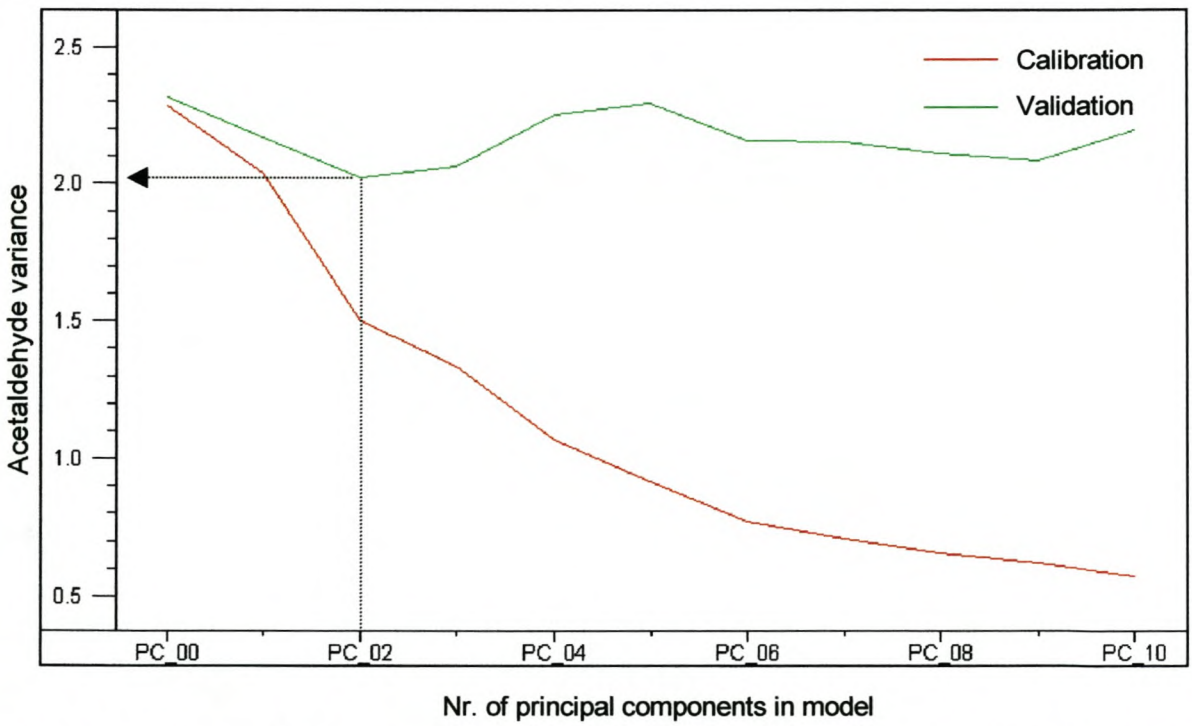

Figure 16. The residual variance plot obtained for the acetaldehyde calibration and full cross validation models of brandy base wine.

Total sulphur dioxide

The very limited range of the total sulphur dioxide values (18-20 $mg.L^{-1}$) prevented the development of a calibration model for the dataset. Attempts could be made in future to evaluate the discriminatory abilities of FT-NIRS with wine samples that contain no total sulphur dioxide and samples that do contain sulphur dioxide.

Conclusions

FT-NIRS provides a viable method for the measurement of the alcohol contents in brandy base wine. Promising correlation statistics were obtained for the total acid contents, volatile acidity, pH and total phenol contents of the brandy base wine. The RER values of these models, however, indicated that the accuracy of these models were not suitable for reliable and robust measurements on future samples. The biggest culprit for the poor accuracy reflected in the FT-NIRS models was probably the reference measurements. Sensitive methods such as HPLC or GC measurement for total acid contents, volatile acidity and total phenols must be employed to obtain more accurate reference values. Improvement of the performance and robustness of the models can be achieved by incorporating wider concentration ranges, more samples and seasonal variability into the data set. Near infrared spectra did not correlate well with the concentration data of reducing sugars and acetaldehyde. For both these constituents, samples with higher concentration levels would be advised before attempting NIRS predictions. Improved sample presentation (automatic sample feeder) is recommended to increase the reliability of the spectral measurements or measurements performed in cells with a path length of at least 1 mm.

FT-NIRS could replace some of the conventional, time-consuming methods that are currently used to asses the quality of brandy base wine. As FT-NIRS requires no sampling preparation and does not generate any chemical waste, it is an extremely convenient and non-hazardous method to use.

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CHAPTER 5

DISCRIMINANT ANALYSIS OF THREE-YEAR OLD, UNBLENDED SOUTH AFRICAN BRANDY WITH NEAR INFRARED SPECTROSCOPY

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Summary

Near infrared spectroscopy (NIRS) shows great potential to successfully discriminate brandy samples in terms of their sensorial classification status. In this study, for the first time, it was found that brandy class membership, obtained through sensorial evaluation, could be predicted successfully with NIRS. Two different types of instruments, a Fourier transform near infrared spectroscopy (FT-NIRS) system with a scanning range between 700-2500 nm and a diode array (DA) system with a scanning range between 500-1700 nm were used. Brandy samples from the 1999, 2000 and 2001 seasons were analysed in this study. Principal component analysis (PCA) of all the spectra revealed clustering into seasonal groups. Classification of the brandy samples using soft independent modelling of class analogy (SIMCA) for the separate seasonal datasets produced the highest correct and the lowest incorrect classification results. In the 1999 dataset, only class 1 (measured with diode array) produced successful classification results. Good classifications (90.9%-100% for FT-NIRS; 71.4%-72.7% for DA) were obtained for the 2000 data. Excellent predictions (100%) were obtained for 3 of the 4 classes within the 2001 FT-NIRS dataset. The 4th class overlapped strongly with one of these classes, probably explaining the poorer classification success. Reasonable classifications were also obtained for the 2001 DA measured dataset. PCA score plots verified the results by revealing distribution of the samples according to class analogies. Poor classification results were obtained with the combined datasets. Overall, the smoothest and hardest classes within the datasets had the strongest predictive potential with NIRS. FT-NIRS measured data obtained the highest correct classification rates, but also classified more false positives than the DA spectra. The 2000 and 2001 data obtained overall better classification results than the 1999 data.

Introduction

Potkettle brandy is distilled through a double distillation process in a discontinuous fashion in copper kettles (Weitz, 2001). The distillation process involves two phases, where the first involves the distillation of 9-12 percent alcohol per volume wine (% alc/vol) to crude-brandy (30% alc/vol). The second distillation process involves the distilling of the crude-brandy to potkettle brandy with an alcohol content of no more than 75% alc/vol. The first distillation can be regarded as a concentrating process of ethanol and the secondary fermentation products that are separated from the non-volatiles and most of the water. These secondary fermentation products include acetaldehyde, ethyl acetate and fusel oils (Weitz, 2001). The second distillation is a slow process (10 to 12 hours) and the product is refined by distilling the crude brandy into three fractions (Anonymous, 1994; Weitz, 2001). These fractions are called the first flow, heart and faints respectively. The first flow is collected within the first twenty minutes of the process and constitutes only about one percent of the original volume in the kettle, containing lots of aldehydes and precious esters (Anonymous, 1994; Weitz, 2001). The heart is distilled until the alcohol percentage of the distillate reaches 50 to 55% v/v and constitutes about one third of the original potkettle volume. The faints contain large amounts of fatty acids as well as about one third volume of the original distillate. The faints and the first flow are redirected to a new feed of first distillate and undergo another second distillation (Anonymous, 1994; Weitz, 2001).

Maturation in French oak barrels for a minimum period of three years helps to obtain a smooth, amber coloured product, which is pleasant to the palate and rich in bouquet and flavours (Peuch & Moutounet, 1992; Anonymous, 1994; Weitz, 2001). During the maturation process, a number of parallel processes occur that modify the composition of the original distilled spirit. Substantial quantities of phenolic and carbohydrate compounds are extracted from the wood and may undergo further change in the aqueous alcohol of the maturing beverage (Piggot *et al.*, 1992).

Emerging from the maturation process is a considerable variety of amber-coloured beverages, each with a different character, flavour and depth of colour (Anonymous, 1994). Blending and correcting is necessary to obtain a product and brand of established and recognised character. The matured brandy is classified before blending based upon the sensory quality of the matured distillate, which can be described as a combination of fullness, softness and taste, as well as flavour

intensity (Weitz, 2001). The exact cause or specific components responsible for these differences are not know, but it has been suggested that certain long chain fatty acids could play the most important role (Steger, 2001). In South Africa, commercial lower cost brandies are diluted to 43% alc/vol with distilled water after blending and bottled. At least 30% of the alcohol content must originate from three-year old potkettle brandy and the rest from neutral grape spirit (not more than 70%). Liqueur brandies are matured for 5, 10, 15 or more years and the oak-matured brandy content range between 35 and 95 percent (Anonymous, 1994). Liqueur brandies are bottled at 38% alc/vol.

In previous studies using NIRS, subjective measurements like pea sensory quality prediction (Martens & Martens, 1986), sensory profile of black tea (Hall *et al.*, 1988), chocolate sensory analysis (Davies *et al.*, 1991), rice taste evaluation (Kawamura *et al.*, 1997), internal quality of fresh clingstone peaches (Van Zyl, 2000) and wine quality grading (Dambergs *et al.* 2001) have been successfully predicted.

SIMCA (soft independent modelling of class analogy) is a chemometric procedure where the raw spectra are compressed by means of principal component analysis (Downey & Beauchêne, 1997). Classes are modelled independently of each other and the cluster models treat new samples separately. An assessment of class membership is made on the basis of an F-test, thus providing a measure of certainty which may be attached to each measurement. SIMCA is reported to have advantages in the separation of very similar materials (Downey & Beauchêne, 1997). Reported applications of SIMCA to food classification using NIR spectra are few in number due to lack of successful results (Downey, 1996). SIMCA has, however, been successfully applied to the diagnosis of mastitis in dairy cows using the near infrared spectra of milk, blood and urine samples (Tsenkova & Atanassova, 2002).

Objective

The classification of unblended brandy is currently performed subjectively by a trained sensory panel. The objective of this study was to develop a NIRS method to classify different classes of unblended, three-year old brandy produced by a commercial distillery objectively. Two different types of NIRS instrumentation, a Fourier transform near infrared spectrophotometer and a diode array (DA) system were compared. The study investigated the predictive abilities of SIMCA models on the separate and combined datasets of three seasons.

Materials and methods

Brandy samples

One hundred and ninety one samples of unblended three year-old rebate brandy with differing alcohol contents were obtained from Distell in Stellenbosch, South Africa over a period of three seasons and stored at low temperature in a dark area untill the NIRS analysis was performed. Each sample was subjected to sensory evaluation, after the three-year maturation period had expired, by a trained panel at the laboratories at the distillery. The samples were diluted to 20% v/v with distilled water and classified into one of five classes, depending on the structure, taste, body and softness of the brandy. Due to limited samples and a very slight difference between the two smoothest classes, the samples were grouped together as one class, noted as class 1. Class 4 denoted samples belonging to the hardest, most "wooded" group. Class 2 and class 3 indicated the samples lying between class 1 and class 4 in terms of their smoothness. Class 2 would therefore be smooth but a bit harder than class 1, and class 3 would be smoother than class 4, but slightly harder than class 2. The distribution of samples in the dataset is presented in Figure 1.

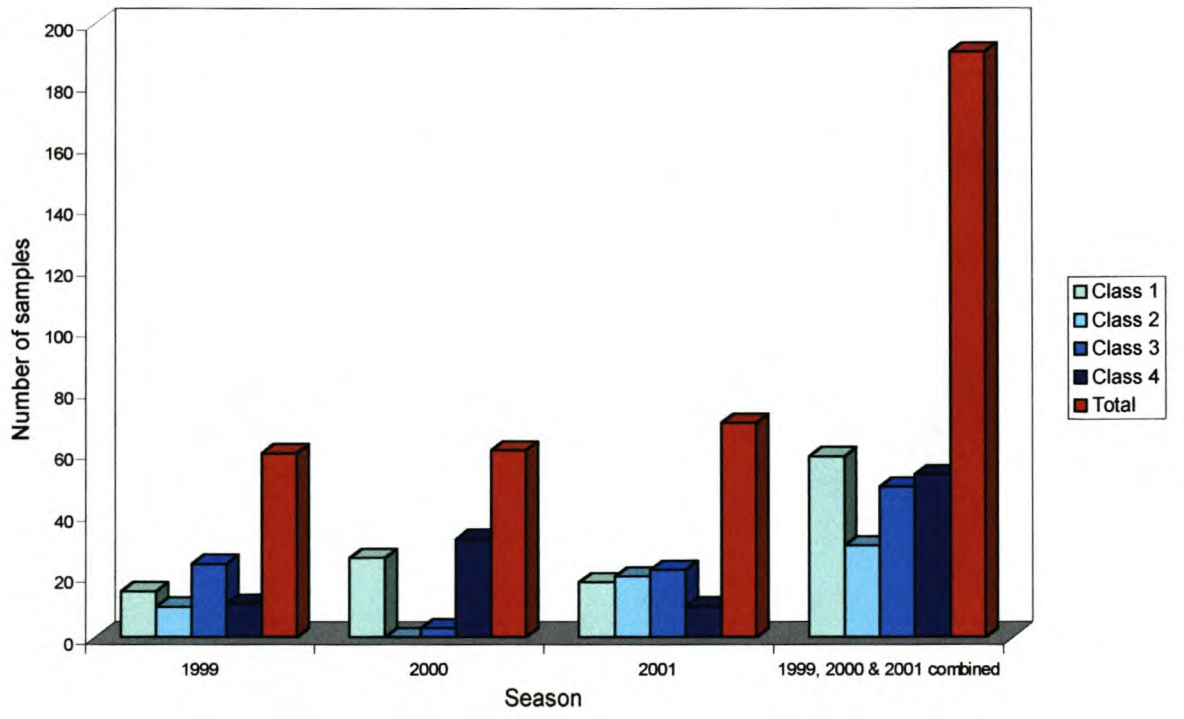

Figure 1. Column graph of the distribution of the brandy samples in terms of class membership and seasonal origin used for the SIMCA modelling.

Spectral analysis

The samples were diluted to 43% alc/vol with distilled water before collection of the spectra to standardise the samples in terms of their water and alcohol contents. Near infrared spectra were recorded in transmittance mode at 2 nm intervals using a Perkin-Elmer Spectrum Identicheck^{TM} 2.0 FT-NIR system. The spectra were collected in the wavelength range between 700 and 2500 nm, with a 16 scan sequence at a resolution of 8 cm^{-1}. The liquid samples were presented in a 1 mm path length Quartz UV/VIS Spectroscopy cell (Perkin-Elmer corp., Norwalk, CT., U.S.A.).

Near infrared spectra were also recorded at 5 nm intervals using a Perten DA 7000 Flexi-Mode NIR/VIS Spectrophotometer (Perten Instruments AB, Huddinge, Sweden). The spectra were collected in the wavelength range between 500 and 1700 nm in duplicate and averaged against the background. A background (black metal container) was collected and the samples presented in a borosilicate-glass petri dish with a rdius of 45 mm, containing 90 ml of liquid placed onto the black metal container.

Chemometric operation

Spectra were exported from the Perkin Elmer *.sp format as ASCII files, whilst spectra from the Perten *.db format were exported and converted via a JCAMP protocol. Principal component analysis (PCA) was performed using SIMCA-P software (ver. 8.00; Umetrics, Sweden). Soft independent modelling of class analogy (SIMCA) models was used for qualitative analysis, i.e. classification of the samples according to class membership. The examined methods for data pretreatment included multiple scatter correction (MSC) and the standard normal variate (SNV) algorithm. All samples were divided into a calibration set (two thirds of the samples) and a validation set (one third of the samples). These samples were chosen to be representative of the entire dataset.

During the first trial, the samples were divided into seasonal datasets (Figure 2) and SIMCA models were developed with the calibration set for each class within each season. Figure 3 illustrates the calibration and prediction testing process. SIMCA modelling of class 1 is used as an example. Prediction testing of the SIMCA model was done on validation and test samples within each specific season. The validation samples refer to the selected samples from each class that were separated

from the calibration set and used as an independent prediction set. The additional test samples refer to the remaining samples in each dataset that did not belong to the class used in the calibration. The same procedure was repeated for each class within each season. The results were reported as "correct classification", i.e. percentage of the samples from the calibration and validation sets that were correctly classified as members of the specific class, and "incorrect classification", i.e. percentage of samples from the additional test set that were incorrectly classified as members of the specific class (i.e. false positives). This was done to test the discriminatory abilities of the models.

During the second trial, the combined seasonal datasets of 1999, 2000 and 2001 were used (Figure 2) and SIMCA models were developed with the calibration set (that contained samples from all three seasons) for each class. The same procedure as shown in Figure 3 (SIMCA modelling of class 1 is used as an example) and explained in the previous paragraph, were repeated.

Factor analyses, i.e. principal components that describe the variations in the spectral data were utilised. The software determined the optimal number of principal components on the basis of a residual variance measure. Probability of membership to a class was made at the 5% significance level.

Results and discussion

In this study, no quantitative determinations were made as in the case of ethanol determinations in wine for example, where specific wavelengths represent certain functional groups. Models were developed purely on subjective classification data. Spectral pre-treatments (multiple scatter correction and standard normal variate transformation) were employed on the data measured by both instruments, but the best classification results were obtained with the untreated spectral data. Different wavelength regions were also investigated, but the best classification results were obtained with both instruments where the entire available spectral region were utilised. The absorption spectra for the FT-NIRS and DA measurements are shown in Figures 4 and 5.

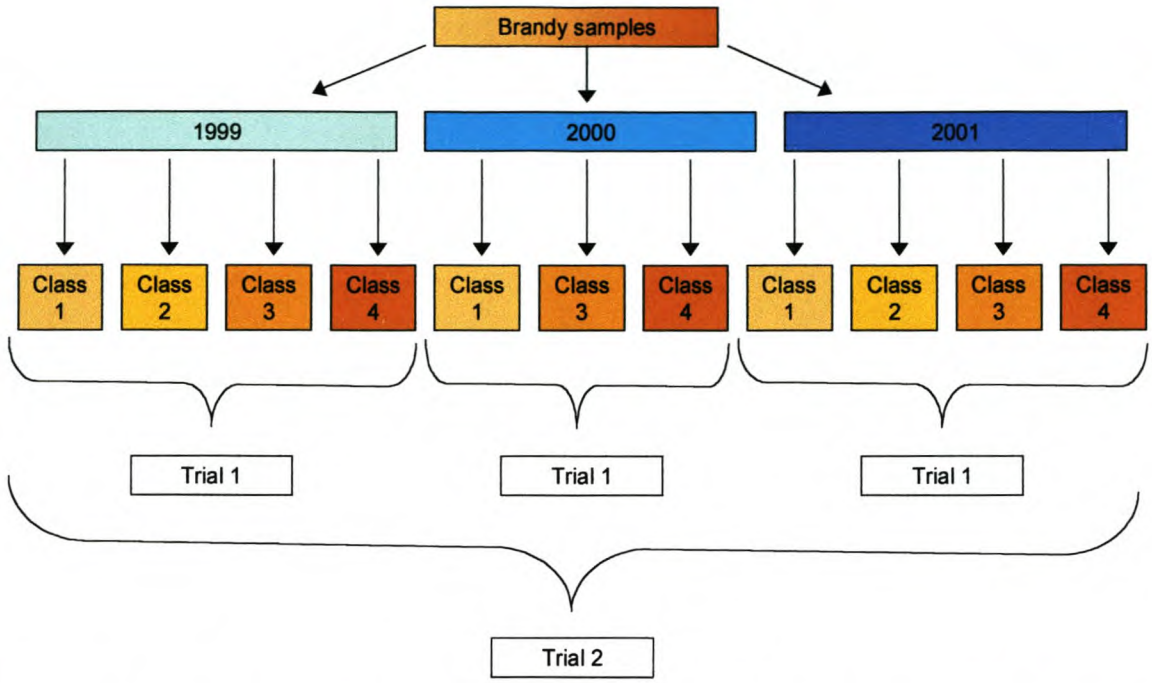

Figure 2. Schematical diagram of the division of brandy samples according to seasonal and class distinction for SIMCA modelling and prediction testing.

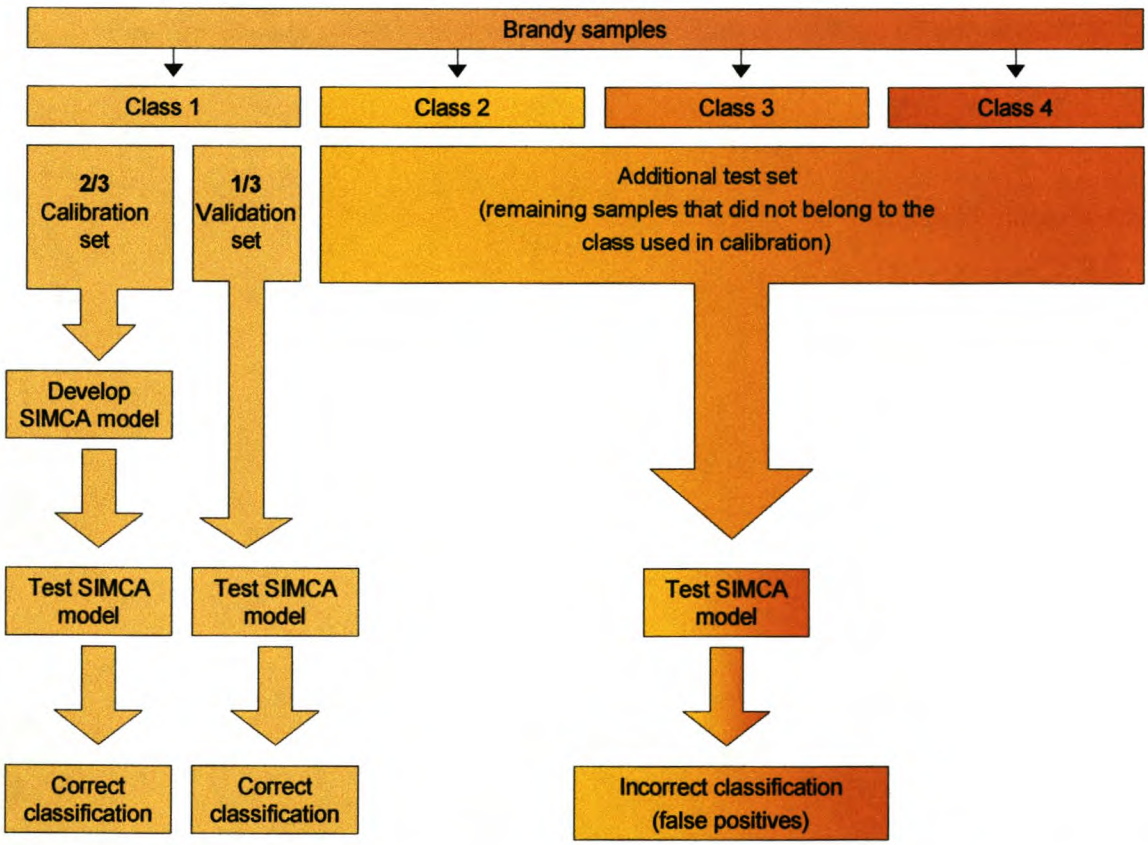

Figure 3. Schematical diagram of the SIMCA modelling and prediction testing

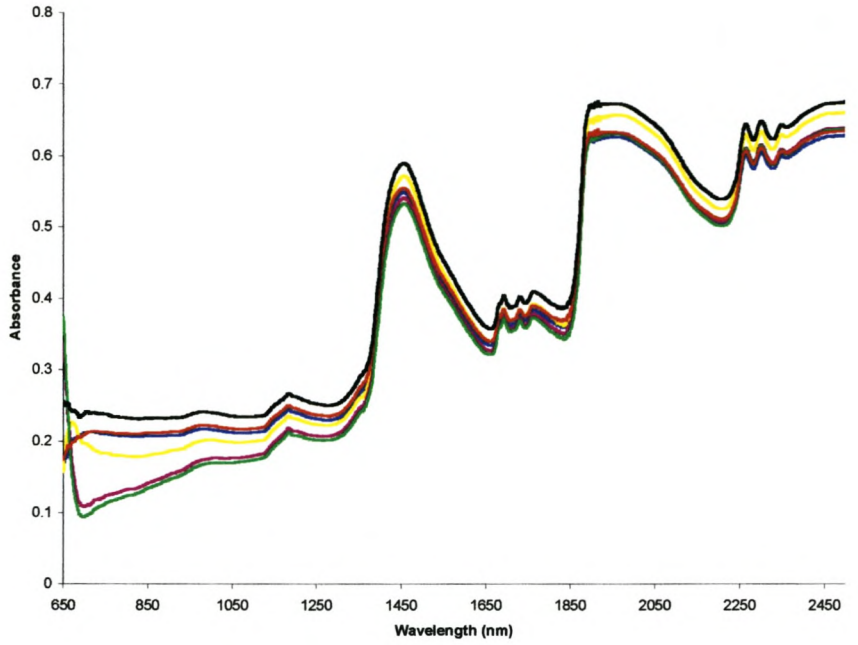

Figure 4. Absorption spectra of unblended brandy measured with a Perkin-Elmer FT-NIRS system.

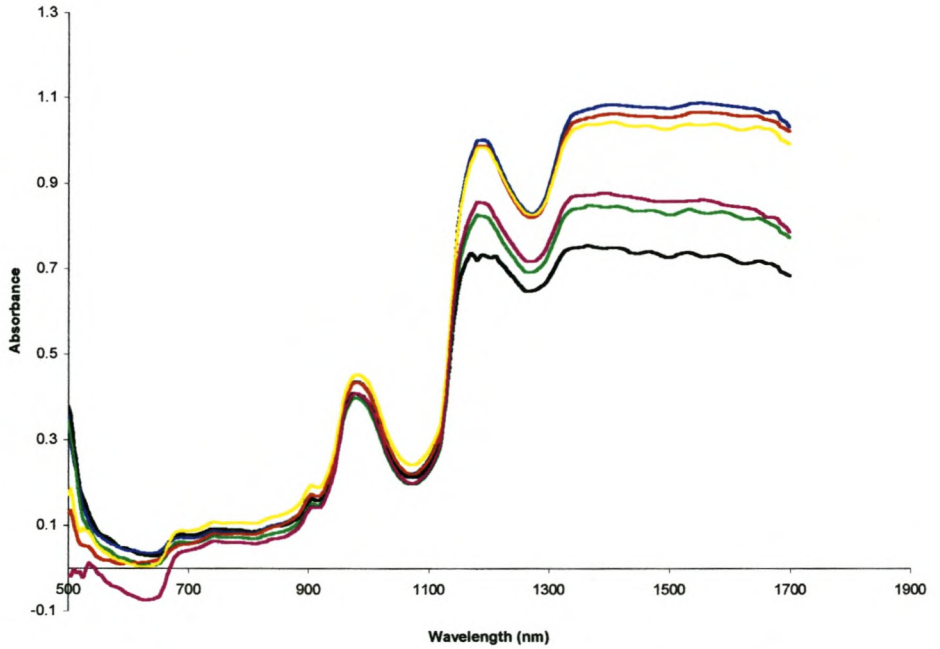

Figure 5. Absorption spectra of unblended brandy measured with a Perten Diode Array spectrophotometer.

Principal component analysis

Principal component analysis score plots of the spectra showed the distribution of the samples within seasonal and class context. Figures 6 and 7 show the seasonal distribution of the samples as revealed through PCA of the near infrared absorbency measurements made with a FT-NIRS instrument and a DA instrument respectively. In both cases, the samples from the 2001 season separated from the 1999 and 2000 seasons, with those measured with the FT-NIRS instrument being more sensitive to the seasonal variation. These score plots suggested that the major variation in the spectral data had a strong relationship to season, and therefore necessitated separation of the data sets into seasonal sets. The differences in sample presentation between the two instruments could have played an important role regarding the sensitivity of the classification abilities of the instruments. The first component of the Fourier transform near infrared spectral data set explained most of the total variation (96.6%) whilst in the diode array spectral data set, the first (53.4%) and second (43.4%) components explained most of the variation. These score plots suggested the need to separate the seasonal data sets and perform classification on each individual seasonal datasets rather than on the combined datasets of all three seasons.

In Figure 8, PCA of the 1999 spectral dataset analysed with FT-NIRS did not reveal any distinct clustering. In Figure 9, however, the same sample set measured with the diode array system revealed distinct clustering of the samples according to class analogy. Figures 10 and 11 show clustering according to class analogy for the samples in the 2000 dataset, as analysed by both instrumental measurements. Better separation of the FT-NIRS 2000 data, was achieved in the third and fourth principal components. In the 2001 dataset for both instruments, clustering according to class analogy was achieved in the first two principal components as shown in Figures 12 and 13.

For the combined sample set where the samples from all three seasons were included, PCA of the FT-NIRS data did not reveal a clear distribution pattern (Figure 14). Clustering of the samples according to class analogy could, however, be seen for the data obtained with the diode instrument when the second and third principal components were plotted against each other (Figure 15).

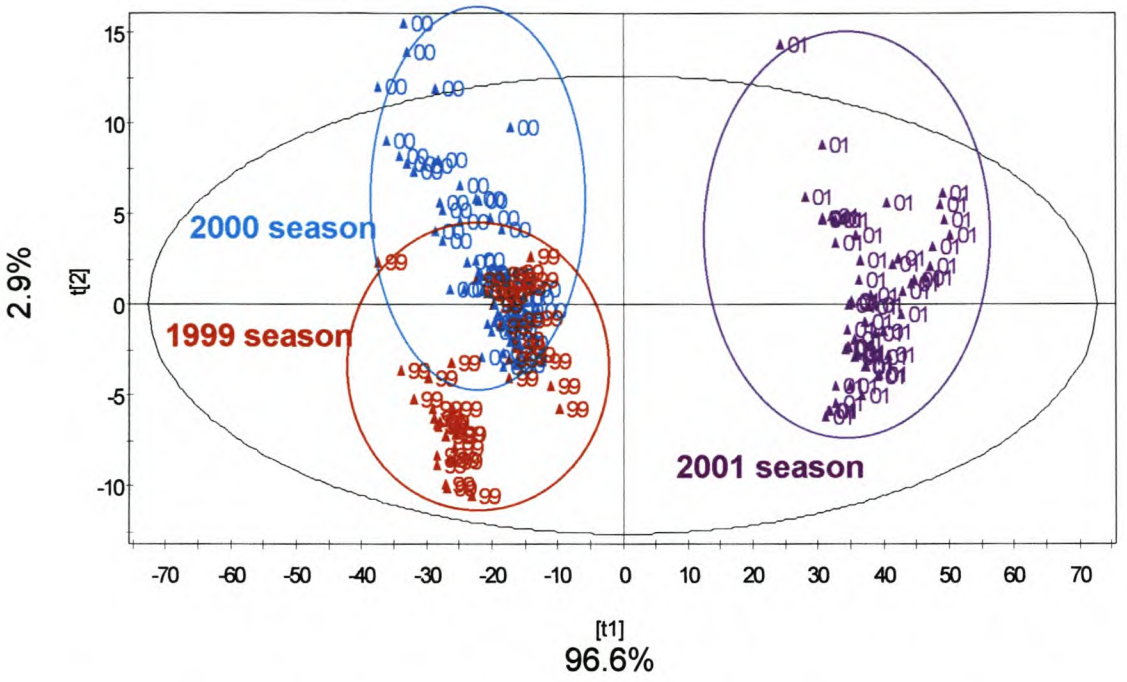

Figure 6. PCA score plot of the first two principal components of brandy spectra measured with FT-NIRS showing the seasonal distribution of the entire dataset.

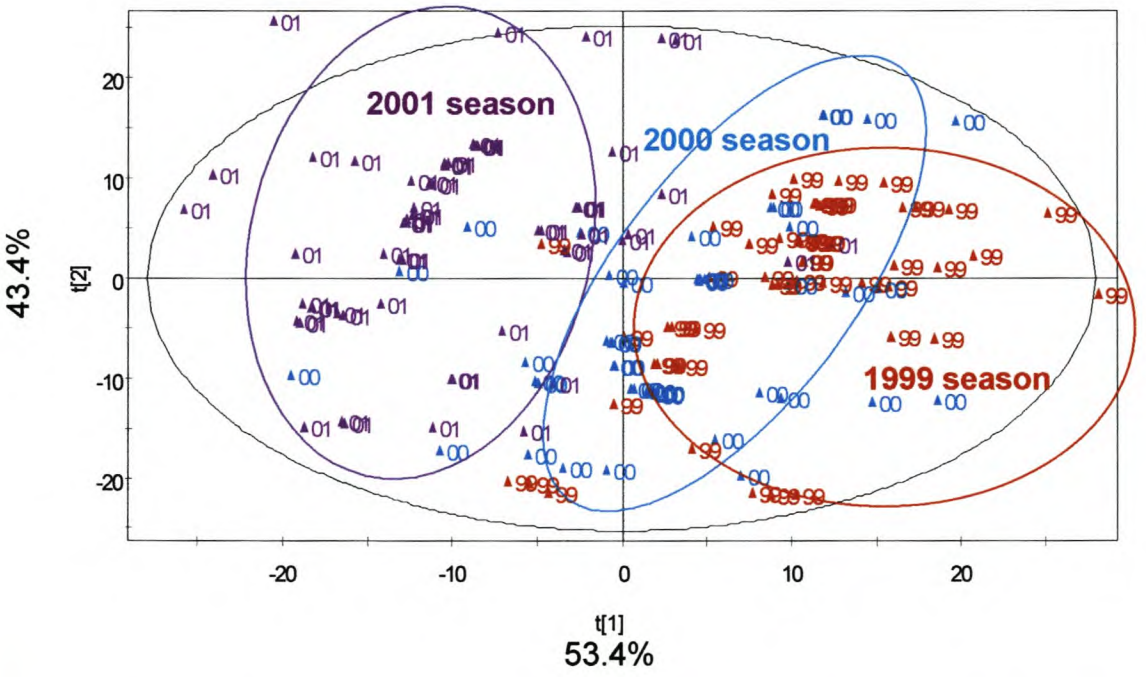

Figure 7. PCA score plot the first two principal components of brandy spectra measured with a DA spectrophotometer showing the seasonal distribution of the entire dataset.

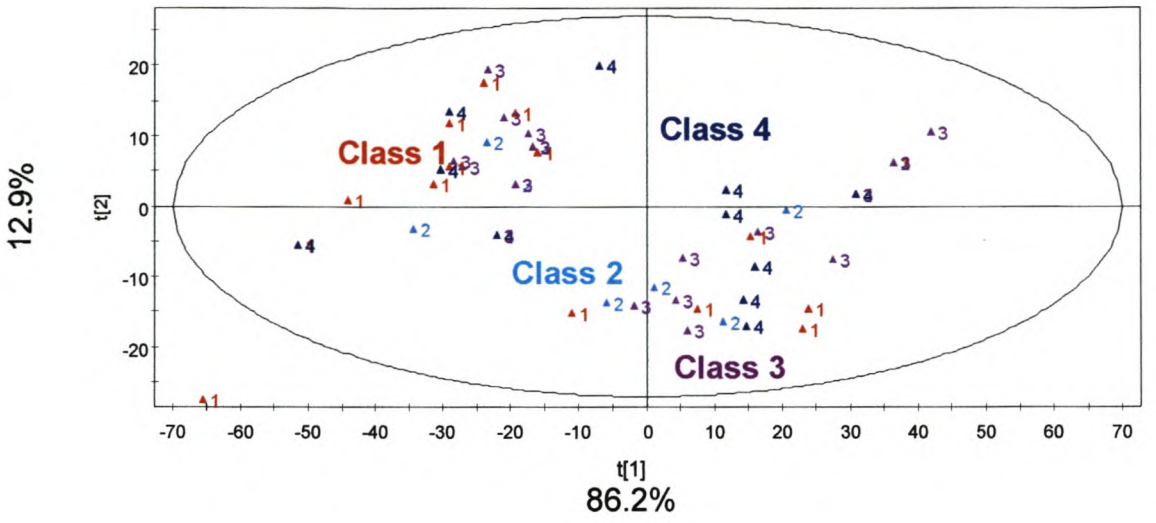

Figure 8. PCA score plot of the first two principal components of brandy spectra measured with FT-NIRS showing the class distribution of the 1999 data.

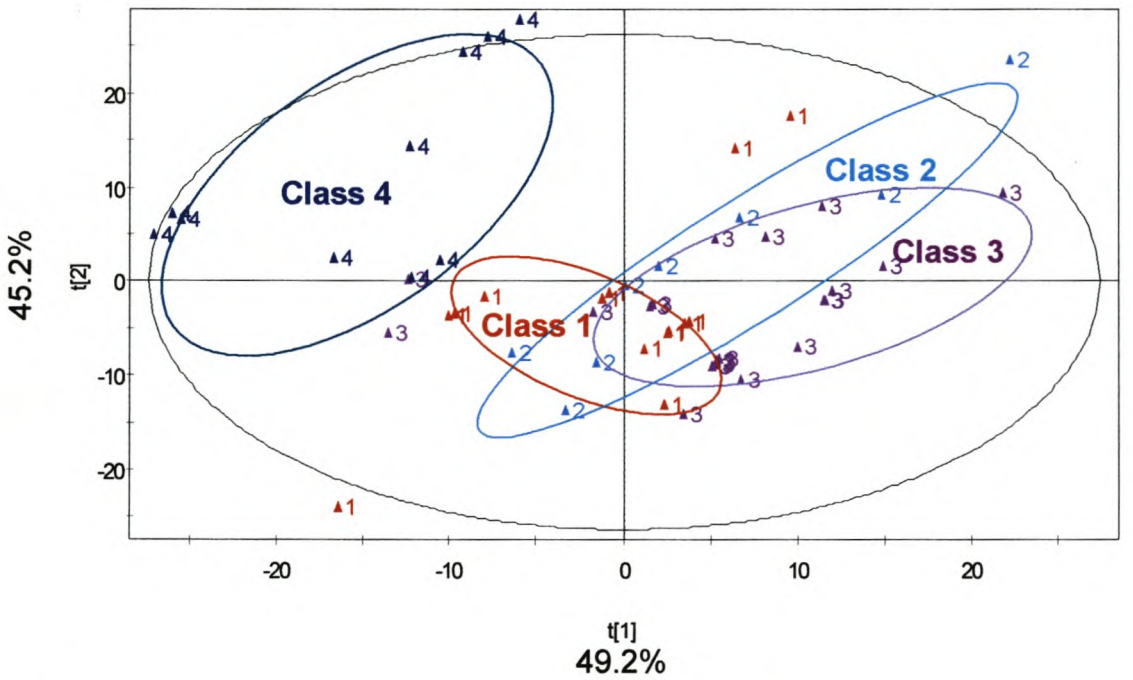

Figure 9. PCA score plot of the first two principal components of brandy spectra measured with a DA spectrophotometer showing the class distribution of the 1999 data.

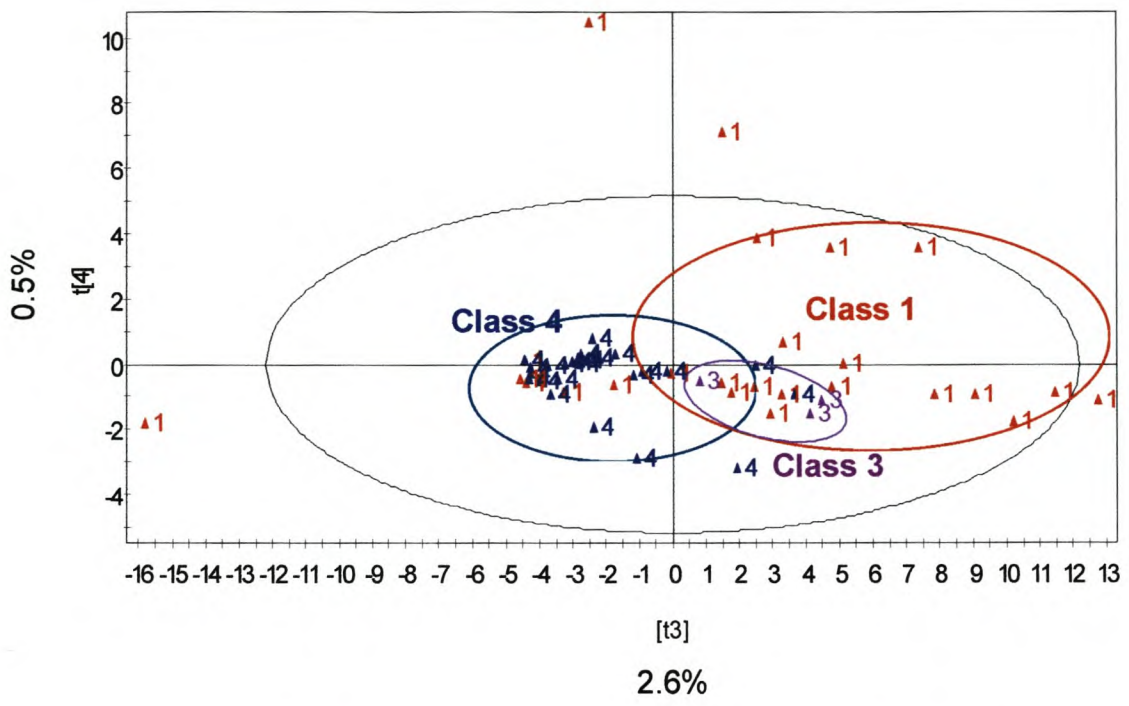

Figure 10. PCA score plot (PC 3 vs. PC 4) of brandy spectra measured with FT-NIRS showing the class distribution of the 2000 data.

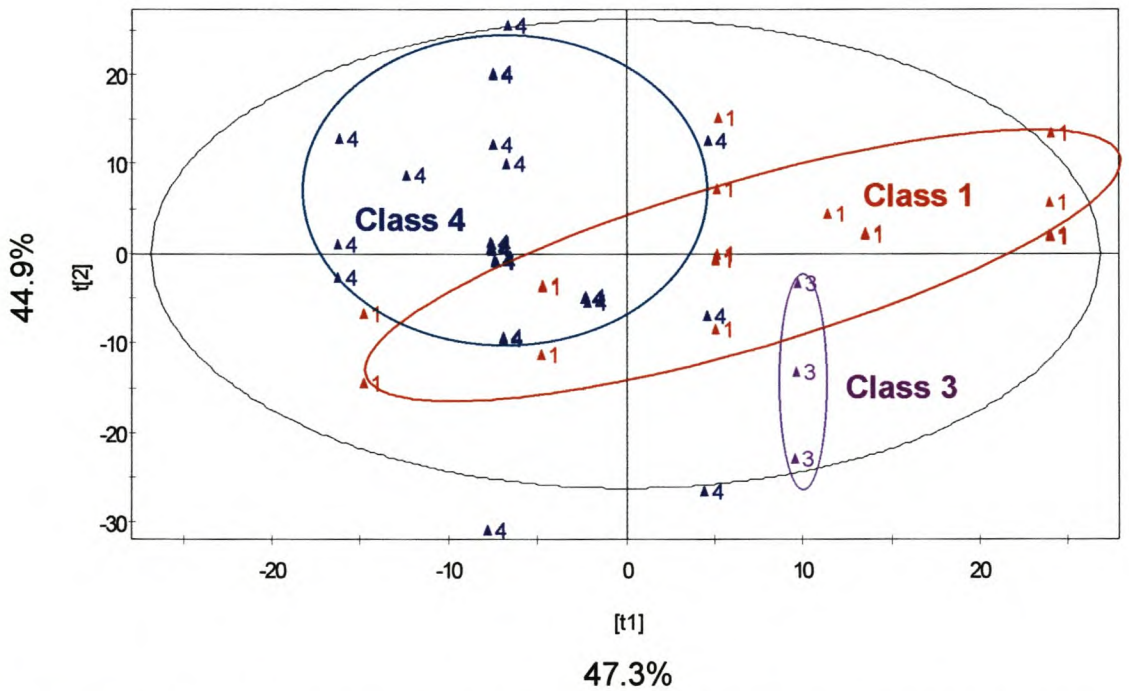

Figure 11. PCA score plot of the first two principal components of brandy spectra measured with a DA spectrophotometer showing the class distribution of the 2000 data.

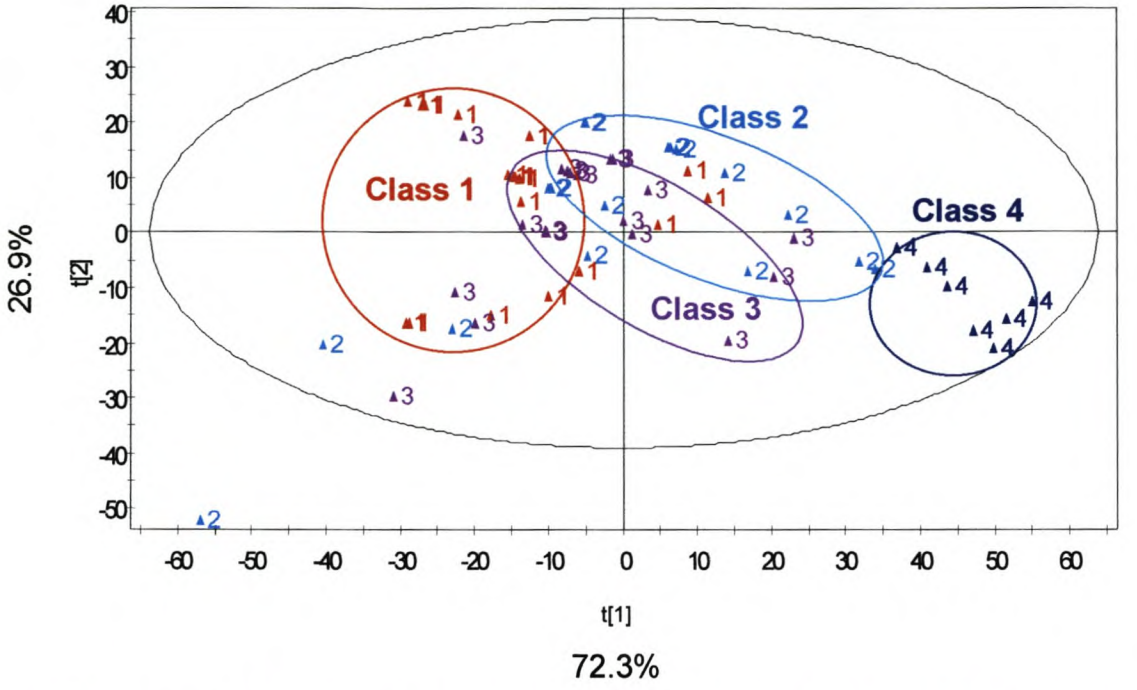

Figure 12. PCA score plot of the first two principal components of brandy spectra measured with FT-NIRS showing the class distribution of the 2001 data.

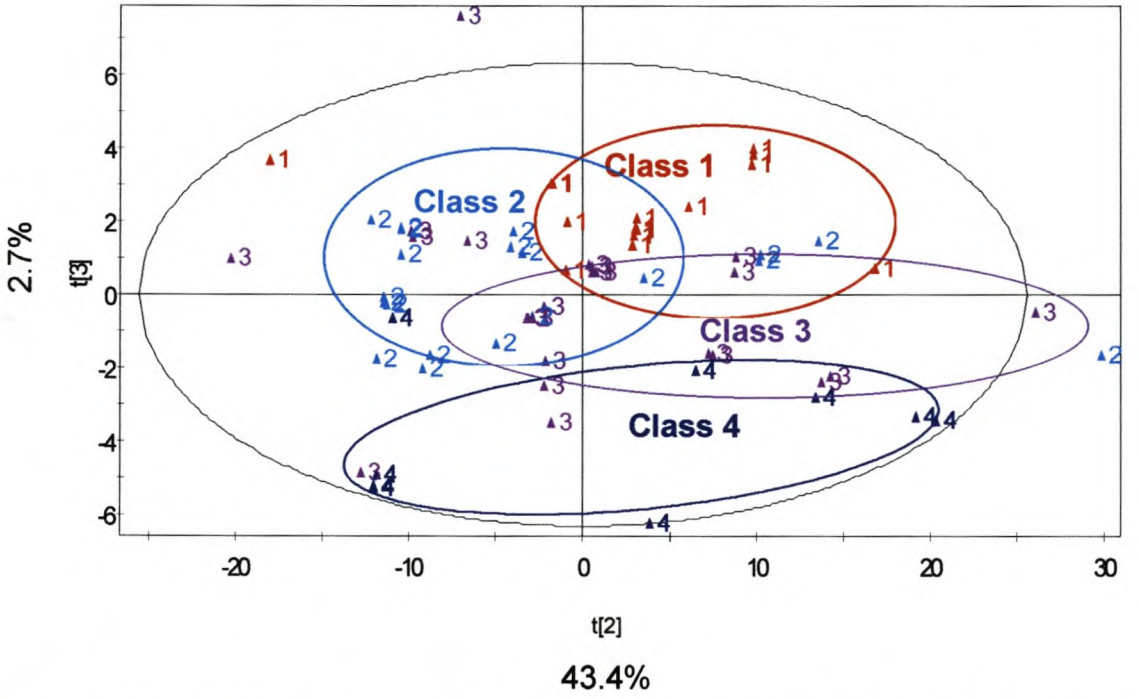

Figure 13. PCA score plot (PC 2 vs. PC 3) of brandy spectra measured with a DA spectrophotometer showing the class distribution of the 2001 data.

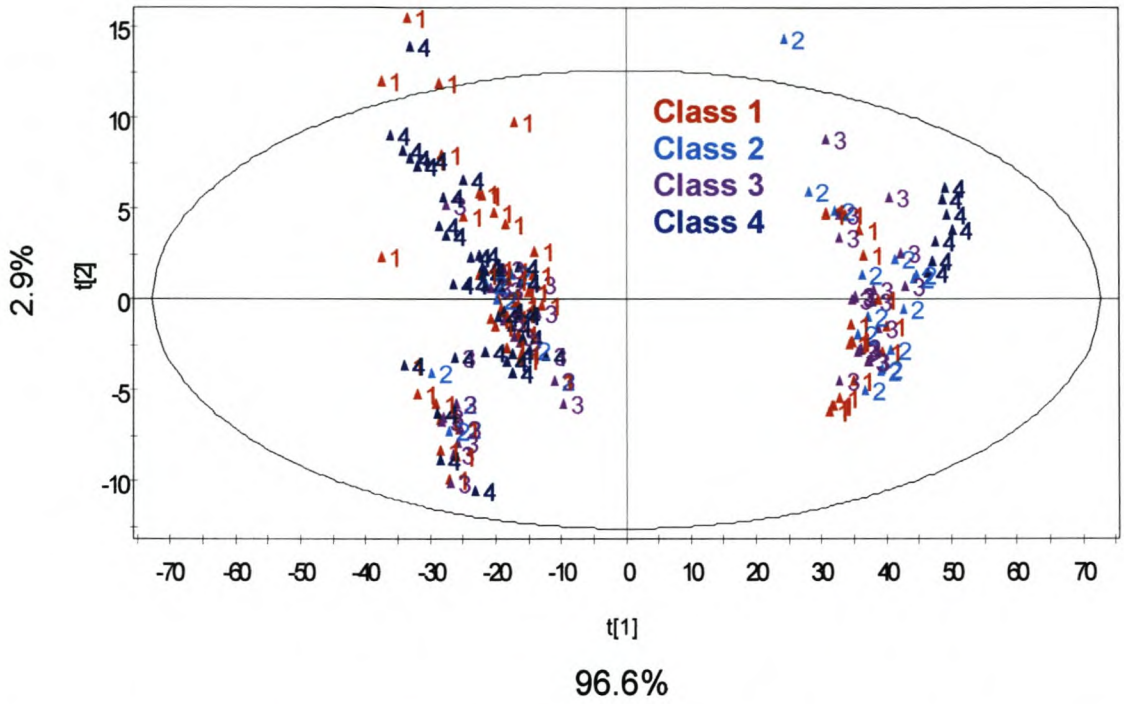

Figure 14. PCA score plot of the first two principal components of brandy spectra measured with FT-NIRS showing the class distribution of the combined data.

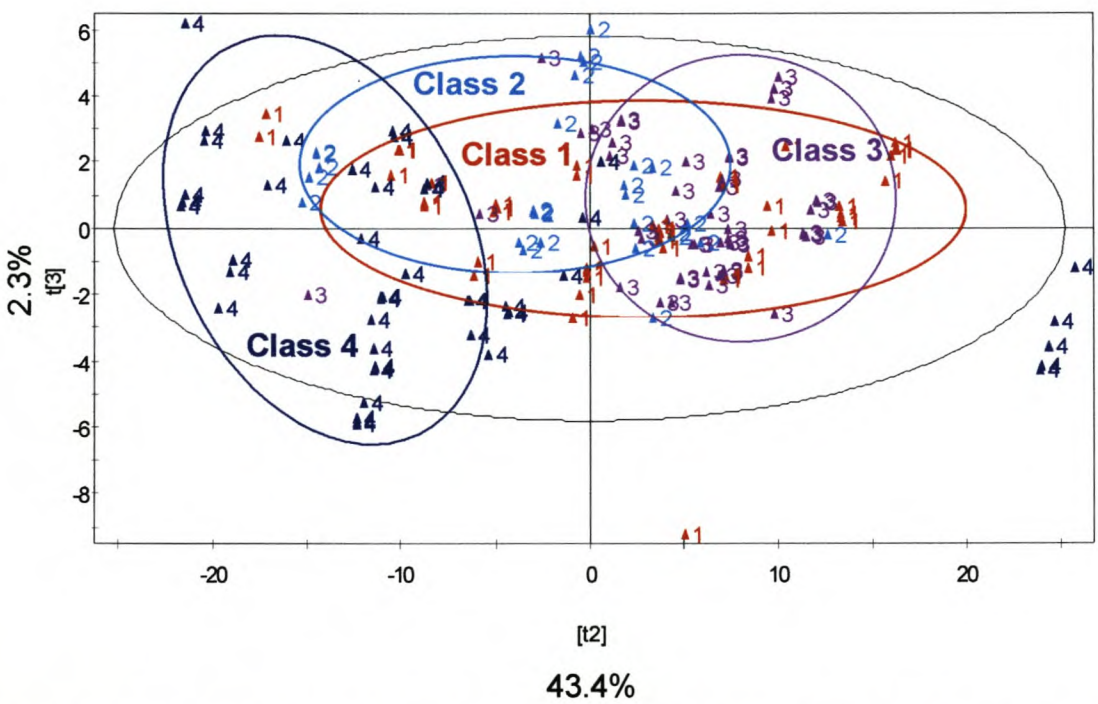

Figure 15. PCA score plot (PC 2 vs. PC 3) of the brandy spectra measured with a DA spectrophotometer showing the class distribution of the combined data.

SIMCA

SIMCA classification of the samples was performed on each seasonal dataset individually. PCA calibration models were developed for each class within each season. The SIMCA classification and prediction testing procedure have been explained previously.

The SIMCA classification results obtained for the individual datasets of 1999, 2000 and 2001 are presented in Table 1. Excellent classification rates were obtained for all the calibration samples in terms of class allocation (71.4-100%). The samples in the validation sets, however, obtained lower positive classification rates (0-100%). An excellent classification rate was obtained for class 1 (100%) of the 1999 data measured with the diode array instrument. These results could, however, not be repeated for the rest of the classes within the 1999 dataset. No incorrect classification results (false positive) were obtained, though. Poor classification results were obtained for the FT-NIR spectra of the 1999 samples with the highest classification rate obtained for class 4 (67.7%). Incorrect classifications occurred in classes 1 (13.3%) and 4 (10.2%). The poor separation obtained for these classes are also seen in the PCA score plot in Figure 8.

Excellent classifications were obtained for classes 1 (100%) and 4 (90.9%) in 2000 measured with FT-NIRS. Two samples in the 2000 dataset were falsely classified as members of class 1. The diode array measured spectra resulted in lower but still very acceptable classification results (71.4% for class 1 and 72.7% for class 2) and no false positives. In the 2001 FT-NIRS dataset, classes 1, 2 and 4 obtained excellent classification results (all 100%). The classification results were lower for the 2001 DA spectra (66.7-71.4%). No incorrect classifications were obtained during the predictions for the class membership of any of the 2001 samples.

Strong overlapping between samples of class 2 and class 3 in the 1999 and 2001 datasets (as seen in the PCA score plots in Figures 8, 9, 12 and 13) resulted in the poorer classification results obtained for these classes. This could be attributed to the slight sensorial difference between the two classes and the complex sample matrix of the brandy with an array of compounds that all influence the sensorial qualities of brandy in various ways. The smoothest and hardest classes (classes 1 and 4) showed the strongest clustering and predictive potential in all cases.

Table 1. Classification results for the SIMCA classifications of three-year old unblended brandy samples within seasonal datasets.

				Correct classification				Incorrect classification	
Classification		Instrument	No. of PC's	Calibration set		Test set		Remaining sample set	
				n	%	n	%	n	%
1999	Class 1	FT-NIRS	4	10/10	100%	3/5	60%	6/45	13.3%
		DA	4	9/10	90%	5/5	100%	0/43	0%
	Class 2	FT-NIRS	3	6/6	100%	2/4	50%	0/50	0%
		DA	2	6/6	100%	2/4	50%	0/48	0%
	Class 3	FT-NIRS	3	13/16	81.3%	5/8	62.5%	0/36	0%
		DA	3	13/16	86.7%	5/8	62.5%	0/34	0%
	Class 4	FT-NIRS	3	7/8	87.5%	2/3	67.7%	5/49	10.2%
		DA	3	5/6	83.3%	0/3	0%	0/49	0%
2000	Class 1	FT-NIRS	5	13/17	76.5%	7/7	100%	2/35	5.7%
		DA	4	14/17	82.4%	5/7	71.4%	0/35	0%
	Class 4	FT-NIRS	5	18/21	85.7%	10/11	90.9%	0/27	0%
		DA	6	16/21	76.2%	8/11	72.7%	0/27	0%
2001	Class 1	FT-NIRS	4	9/11	81.8%	7/7	100%	0/52	0%
		DA	4	10/11	90.9%	5/7	71.4%	0/52	0%
	Class 2	FT-NIRS	4	12/13	92.3%	7/7	100%	0/50	0%
		DA	3	12/13	92.3%	5/7	71.4%	0/50	0%
	Class 3	FT-NIRS	4	14/15	93.3%	4/7	57.1%	0/48	0%
		DA	5	13/15	86.7%	5/7	71.4%	0/48	0%
	Class 4	FT-NIRS	3	6/7	85.7%	3/3	100%	0/60	0%
		DA	2	5/7	71.4%	2/3	66.7%	0/60	0%

The sensorial difference between classes 1 and 4 is more distinct than the difference between either of these classes and classes 2 and 3. The samples in the 2001 dataset obtained the highest positive classification rate and no incorrect classifications for both instruments. No false positive classifications were obtained for any of the diode array datasets.

SIMCA classification of the samples was also performed on the combined datasets of the 1999, 2000 and 2001 seasons and the results are presented in Table 2. When the datasets of the three seasons were combined, poor classification rates were obtained for the FT-NIRS and DA data. High correct classification rates were obtained for both instruments (81.8-100% with FT-NIRS and 72.7-93.3% with DA, but the high rate of false positive classifications revealed the poor discriminatory abilities of the models.

The variation resulting from the evaluation process of the brandies could be the major cause of error in the data. The difficulty to obtain perfect clustering of the data could be attributed to the complex sample matrix and consequent variation of the brandies. The samples may span growing areas and grape varieties, even though the winemaking and aging practises were kept constant. Colour differences that were not linear to the class differences, could also affect the spectral information, as the scanning range overlapped the visible light range (500-700 nm). Classes 2 and 3 should possibly be regarded as one class for NIRS classification purposes. Uniformity in sample presentation must be achieved before comparisons can be made between the instrument types.

Conclusions

Based on its classification power, NIRS and SIMCA appeared to be a promising tool when used in the analysis of unblended brandy. Classification data obtained from the subjective sensorial evaluation of unblended three-year old brandy were successfully correlated to NIRS data. The best prediction results were obtained with untreated spectral data of the separated seasonal datasets. Poor predictions of class membership were obtained with the combined datasets of three seasons' spectral data. This technique could, however, be employed to predict membership of samples to a specific season and thereafter use the specific seasons' models to predict class membership. By adding new datasets from future seasons, a robust classification system can be developed.

Table 2. Results for SIMCA classification of three-year old unblended brandy samples of combined seasonal datasets.

			Correct classification				Incorrect classification	
Classification	**Instrument**	**No. of PC's**	**Calibration set**		**Validation set**		**Remaining sample set**	
			n	%	n	%	n	%
Class 1	FT-NIRS	3	35/38	92.1%	18/21	85.7%	114/132	86.4%
	DA	4	29/38	76.3%	18/21	85.7%	55/132	41.7%
Class 2	FT-NIRS	3	15/19	78.9%	9/11	81.8%	111/161	68.9%
	DA	3	16/19	84.2%	8/11	72.7%	42/161	26.1%
Class 3	FT-NIRS	3	27/34	79.4%	14/15	93.3%	78/142	54.9%
	DA	4	26/34	76.5%	14/15	93.3%	62/142	43.7%
Class 4	FT-NIRS	2	31/36	86.1%	16/16	100%	28/139	20.1%
	DA	5	25/34	73.5%	15/18	83.3%	71/139	51.1%

Advances concerning automated liquid sample presentation of NIRS instrumentation could greatly enhance the spectral acquisition of the data and probably improve the predictive abilities of the modelling. Due to the slight differences between the samples and the variation induced through the subjective classification process, distinct clustering or grouping of the different classes could not be expected. Future studies aiming at investigating the specific components responsible for the sensorial differences, could be applied to great advantage with NIRS classification tools if these compounds are present in concentrations above the near infrared detection limit. These results warrant further investigation and may provide valuable insight into the character of brandy and the differences found amongst the different styles. NIRS and SIMCA offers a rapid and objective method to classify brandy samples according to its sensorial classification status.

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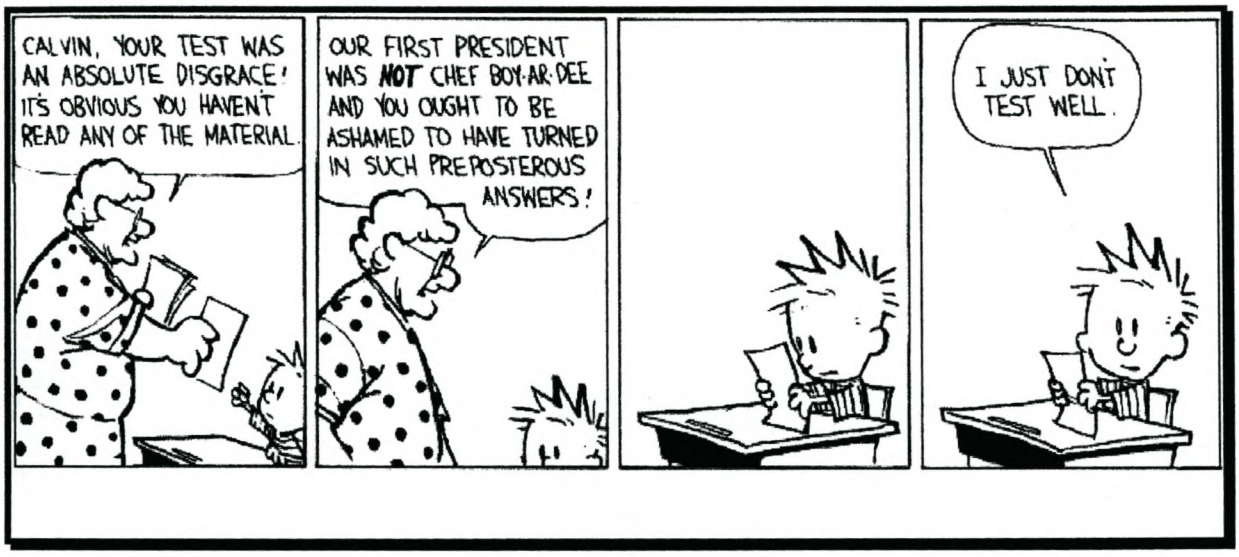

CHAPTER 6

EVALUATION OF REFERENCE DATA USED FOR NEAR INFRARED SPECTROSCOPY CALIBRATIONS

CHAPTER 6

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Summary

Having accurate reference data in the calibration set is essential in near infrared spectroscopy (NIRS). The precision (usually decided by blind duplicate determination) of the reference data serves as the performance target for correlation-based near infrared analysis. Results of a study of the measurement of alcohol contents, volatile acidity and total sulphur dioxide concentrations in distilling wine by two independent laboratories and an instrumental measurement are reported. Analysis of variance showed that there was a statistically significant difference ($p<0.05$) between the three sets of values obtained by the laboratories and the Foss Winescan for alcohol and volatile acid measurements. Further post-hoc testing revealed statistically highly significant differences between both laboratories ($p = 0.006$) and between the instrumental method and laboratory B ($p = 0.004$) and a very highly significant difference between the instrumental method and laboratory A ($p<0.001$) for the volatile acid analysis. For the alcohol analysis of the distilling wine the measurements performed by laboratory A differed significantly from those performed by laboratory B ($p = 0.013$) and by the Foss Winescan ($p = 0.049$). A paired t-test revealed a statistically very highly significant difference ($p<0.001$) between the two laboratory measurements for total sulphur dioxide in distilling wine. The results of this study, suggested the need for a thorough study and probably a proficiency test to be conducted amongst winery laboratories in the Western Cape.

Introduction

According to Osborne *et al.* (1993), the major limitation of near infrared spectroscopy (NIRS) in food analyses concerns its dependence on often less precise chemical methods of analyses. Having accurate reference data in a calibration set is, however, essential. The precision of the reference data (standard error of laboratory)

serves as the performance target for chemometric near infrared analysis methods (Wetzel, 1998).

Calibration of an instrument involves drawing a comparison between a measured quantity and a reference method (Prichard *et al.*, 1995). NIRS analysis is mainly dependent on the development of an empirical linear relationship in which the concentration of a constituent is related to optical measurement, usually expressed in absorbance or, in the case of reflectance measurements, log 1/reflectance (Wetzel, 1998). The chemometric operations that are used to relate the optical measurements to quantitative data aid the development of a calibration model that can be used for future predictions of a specific constituent concentration. Least squares regression is the standard method for calibration under conditions where a chemical method has been used to determine the reference values of a specific constituent (Mark, 1992). Regression has become the preferred method for two reasons. Only the constituent for which a calibration is required, needs to be determined by the reference method. The reference methods normally contain the largest errors and these errors being placed into the data as the *Y*-variables, creates symmetry between the **x** (spectral data) and *Y*-variables.

Accuracy refers to the closeness of the agreement between the result of a measurement and the true value of the quantity being measured (Prichard *et al.*, 1995). It is, therefore, necessary to know the true compositional values of the samples to determine the accuracy of a specific analysis (Butzke & Ebeler, 1999). This is, however, impossible in most cases unless the samples were specifically formulated and the quantities of specific composites known. A high degree of accuracy is not important for trace analysis where the concentration of the constituent is well below the permitted level (Prichard *et al.*, 1995). In situations where the concentration of a constituent is close to the maximum allowed level, accuracy becomes more important.

Precision is the closeness of a series of replicate measurements to each other (Prichard *et al.*, 1995). Uncertainty can arise from random and systematic effects that can result in possible random or systematic errors. The precision or uncertainty of repeated measurements is estimated by the average standard deviation of a series of replicates (Butzke & Ebeler, 1999). Precision, as is the case with accuracy, becomes more important when the concentration of a constituent is close to the maximum allowed level (Prichard *et al.*, 1995).

Random errors arise as the results of chance variations in factors that influence the value of the quantity being measured, but which in itself is outside the control of the person making the measurements (Prichard *et al.*, 1995). The lack of homogeneity of the raw material could also give rise to random errors (Huitson, 1966). In NIRS, these errors are usually denoted as noise. It is not possible to correct one-off values for random error, but by repeating measurements, such error can be reduced (Prichard *et al.,* 1995). Systematic errors (also referred to as bias) remain constant or vary in a predictable way over a series of measurements (Prichard *et al.,* 1995). This type of error can be corrected for, if the value of the error is known.

Control over laboratory efficiency, accuracy and precision is an important aspect of sound laboratory management. A proficiency testing scheme comprises the regular distribution of test materials to participating laboratories for independent testing (Lawn *et al.,* 1997). The primary function of the scheme is to assist the participants to detect shortcomings in their execution of the test procedures and apply suitable remedial measures to make up for any deficiency.

During 1995, a proficiency trial carried out under the auspices of the Interwinery Analysis Group in Australia, revealed that none of the eighteen laboratories involved in the trial achieved results within 0.3% v/v of the standard with a certified alcohol strength of 11.1% v/v (Weeks, 1995). As a result, a quality control system was put in place. Butzke & Ebeler (1999) found a proficiency of less than 20% for wineries in measuring titratable and volatile acidity in a survey conducted on the analyses of five basic wine measurements (pH, titratable acidity, volatile acidity, alcohol, and residual sugar). The range of reported volatile acidity concentrations varied more than two-fold. For residual sugar measurements, the trial revealed unsatisfactory analysis results with a coefficient of variation of 140%. Not only does this suggest a need for improved laboratory quality management systems, especially with respect to analytical method standardisation and validation, but also for a laboratory proficiency-testing program.

Prichard *et al.* (1995) describes a laboratory quality system as "*a formal structure set up to encompass all aspects of quality in the laboratory*". The International Organisation for Standardisation (ISO) has produced a range of standards and guidance relevant to laboratories (Prichard *et al.,* 1995; Lawn *et al.*, 1997; Butzke & Ebeler, 1999). The ISO 9000 series of quality standards is the most

relevant and simplifies the approach to select a quality product or service provider. Locally, the South African Bureau of Standards (SABS) was created as national body with the responsibility of establishing, regulating and publicising compulsory specifications and standards (SABS, 2001).

Objective

A standard of certified alcohol strength, volatile acidity and total sulphur dioxide concentration was not available to test the accuracy or proficiency of the two laboratories and instrumental method. The aim of the study was only to investigate the differences that were found amongst three different analyses performed independently by three different laboratories (the third laboratory employed an instrumental method) for the measurement of alcohol and volatile acid in distilling wine samples and between two different laboratories for the measurement of total sulphur dioxide.

Materials and methods

A selection of 100 distilling wine samples, representative of the Western Cape region and distributed throughout the wine season of 2001, were collected from Distell, South Africa. The distilling wine samples were analysed on receipt by a laboratory (for the purpose of this study called laboratory A), where after it was analysed at an independent laboratory (for the purpose of this study called laboratory B) and with a Foss Winescan FT 120 spectrophotometer (Foss Electric, Denmark) at the Institute for Wine Biotechnology at the University of Stellenbosch. For the total sulphur dioxide contents, the instrumental determination could not be performed as no commercial calibration was available for total sulphur dioxide in wine. All the analyses for a specific wine sample were performed within 48 hours and the wine was kept at a constant temperature of 4°C. The wine samples were analysed in terms of their alcohol content, volatile acidity and total sulphur dioxide concentrations. Laboratories A and B performed similar chemical analyses methods for the alcohol and volatile acid quantification of the wine samples. Two different chemical methods were employed by the two laboratories for the total sulphur dioxide measurement.

Chemical analyses

Alcohol

The alcohol content of the wine was determined pycnometrically by measuring the specific gravity of the wine and the alcohol-water distillate as described by the AOAC method nr. 920.57 (AOAC, 2000). The analyses were performed in duplicate and the results reported as the averaged value. Laboratories A and B followed the same procedure.

Volatile Acidity

Steam distillation of the sample as described by Gowans (1964) was followed by titration with standardised sodium hydroxide to a phenolphthalein end point and the results reported in $g.L^{-1}$ acetic acid (AOAC, 2000). The analyses were performed in duplicate and the results reported as the averaged value. Laboratories A and B followed the same procedure.

Total sulphur dioxide

Laboratory A measured the sulphur dioxide content of the distilling wine iodometrically by potassium iodate/iodide using the AOAC (nr. 940.20) Ripper method (AOAC, 2000). The analyses were performed in duplicate and the results reported as the averaged value. Laboratory B measured the total sulphur dioxide levels in the distilling wine by means of the modified Monier-Williams aeration method (AOAC, 2000). This method involves the distillation of SO_2 (with nitrogen as a sweeping gas or with air aspiration) from the sample into peroxide and the subsequent titration of the formed H_2SO_4. The analyses were performed in duplicate and the results reported as the averaged value.

Fourier transform infrared (FT-IR) spectroscopy measurements

The Foss Winecan FT 120, which like NIRS is a predictive method, was connected to and controlled by a computer using proprietary software to enable prediction of the samples. The supplied calibration (referred to as the 'DFW calibration, version 1.1') was developed using dry French and Spanish wines. No sample preparation was required and a settled aliquot of about 40 mL wine was presented manually to the instrument using the sampling sipper. The sampling sipper was manually cleansed after every five samples using the Foss rinsing solution and manually zeroed every

60 minutes using the Foss zeroing solution. The samples were scanned in duplicate and six parameters were analysed simultaneously. The parameters analysed were alcohol, pH, volatile acid, total acid, malic acid and lactic acid, but for the purpose of this study, only alcohol and volatile acidity were considered. No calibration was available for the total sulphur dioxide content.

Analysis of variance

Analysis of variance (ANOVA) and post-hoc analysis (Bonferroni tests) were performed on the alcohol and volatile acid reference results, while a paired t-test was performed on the reference results obtained for total sulphur dioxide, utilising Statistica version 6.00 software package by Stat-Soft (Tulsa, OK. U.S.A). Results were reported at the 5% significance level.

Results and discussion

Alcohol

The values obtained for the measurement of the alcohol concentration in the 100 distilling wine samples by two independent laboratories and the Foss Winescan are shown in Table 1. Laboratory A obtained the largest concentration range and the lowest mean. This implicated that a bias effect could not be attributed to the difference between laboratory A and laboratory B and the Foss Winescan. A correlation graph of the three different values obtained for the alcohol measurement of the wine samples are presented in Figure 1. The values obtained from laboratory B were used as standard to plot the other two values against. Both laboratories A and B measured the alcohol strength pycnometrically, which is the EU reference method. Systematic differences amongst the methods could therefore not be blamed for the differences. The ANOVA results (Table 2) revealed that there was a significant difference between the values obtained for the alcohol content of the sample set between the three laboratories ($p = 0.009$). Figure 2 also showed that the results obtained from laboratory B and the Foss Winescan differed only slightly compared to the results obtained from laboratory A. A Bonferroni test (Table 3) confirmed that the difference between the alcohol concentration values obtained from laboratory B and the Foss Winescan were statistically insignificant ($p = 1.00$). Significant differences existed between the values obtained from laboratories A and B ($p = 0.013$), and laboratory A and the Foss Winescan ($p = 0.049$).

Table 1. Results obtained for the measurement of the alcohol content in distilling wine (% v/v) by two independent laboratories and an instrumental method.

	Laboratory A	Laboratory B	Foss Winescan
n	100	100	100
Mean	10.18	10.24	10.23
Min	6.67	7.07	7.02
Max	13.61	13.49	13.64

Table 2. Analysis of variance for the alcohol determinations in distilling wine.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	p
Laboratories	2	0.20	0.10	5	0.009
Error	198	4.21	0.02		

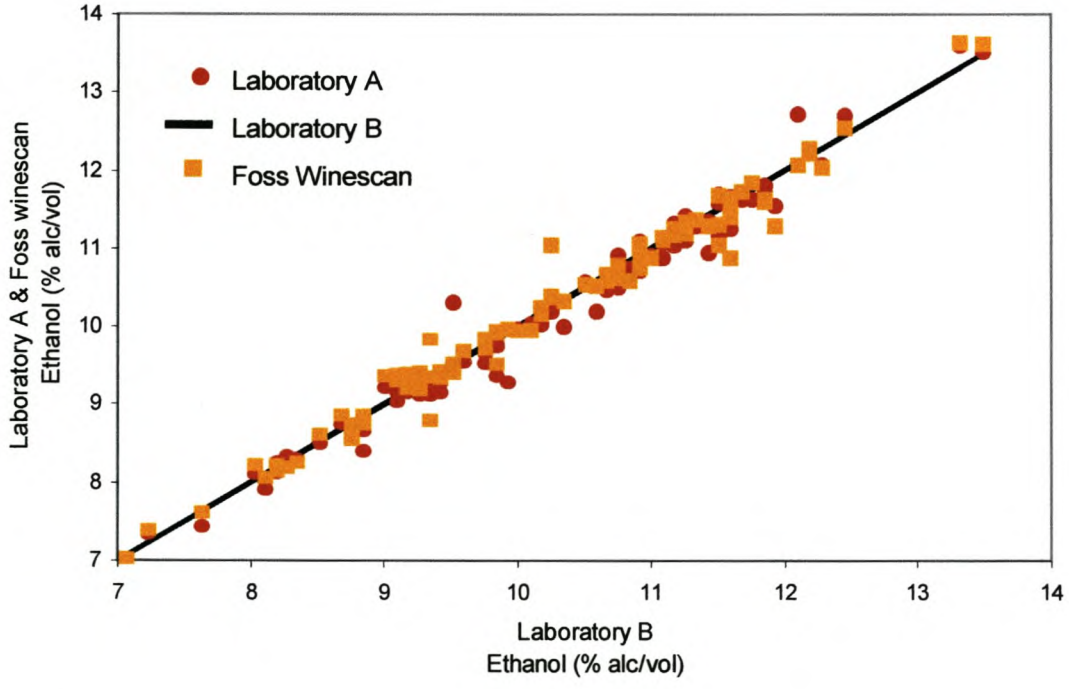

Figure 1. Correlation graph of alcohol concentrations in distilling wine as measured by two independent laboratories and an instrumental method.

Table 3. Probabilities obtained from post-hoc analysis (Bonferroni test) performed on the results obtained for alcohol measurements.

	Laboratory A	Laboratory B	Foss Winescan
Laboratory A	-	0.013	0.049
Laboratory B	0.013	-	1.00
Foss Winescan	0.049	1.00	-

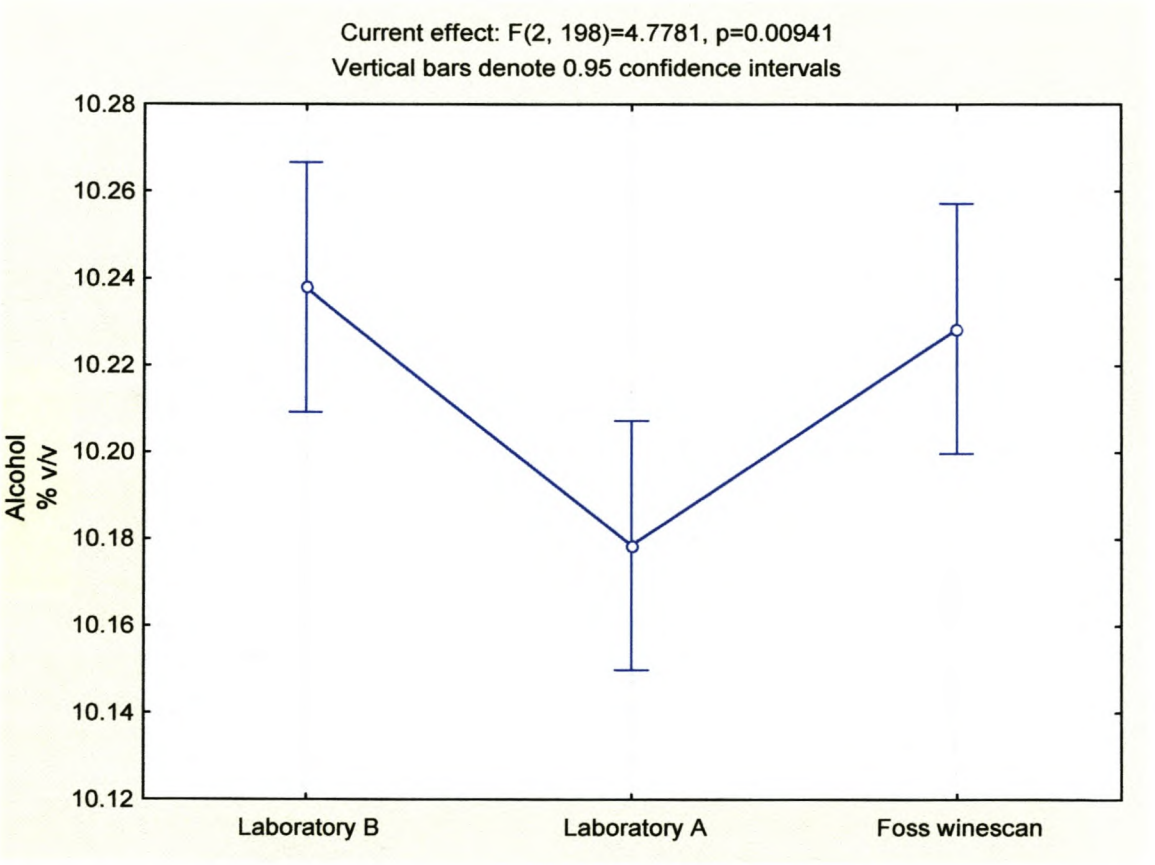

Figure 2. ANOVA plot of the measurement differences obtained for alcohol concentrations in distilling wine as measured by two independent laboratories and an instrumental method.

Volatile acid

The values obtained for the measurement of the volatile acidity in the 100 distilling wine samples by two independent laboratories and the Foss Winescan, are shown in Table 4. The values obtained from laboratory B were used as standard to plot the other two values against. The Foss Winescan had an overall lower concentration range measurement and laboratory A had an overall higher concentration measurement, which can be seen in the bias effect for both measurements in Figure 3. Greater variation occurred in measurements where the concentrations exceeded 1.5 $g.L^{-1}$. The Foss Winescan operates with a calibration package developed on European wines. Wines from the Southern Hemisphere generally differ from Northern Hemisphere wines in that the warmer climate produce wines with lower acidity and higher pH. This could possibly have an effect on the analysis performance of the instrument and explain the strong bias effect. Bias correction of the instrument for South African measurement purposes could possibly solve the problem. Analyses of the wines by laboratory A were performed prior to those (in the course of 24 hours) done by laboratory B and the Foss Winescan. Evaporation of the volatile acids after the analyses by laboratory A could have resulted in an overall lower concentration range. The ANOVA results in Table 5 revealed that there was a significant difference ($p<0.001$) between the values obtained for the volatile acid content of the sample set between the two laboratories and the Foss Winescan. In Figure 4 the large differences between the results from all three measurements are shown. Post-hoc analysis (Bonferroni test) in Table 6 confirmed that the differences between the volatile acid values obtained from all three measurements were statistically significant ($p<0.05$).

Table 4. Results obtained for the measurement of volatile acidity ($g.L^{-1}$) in distilling wine by two independent laboratories and an instrumental method.

	Laboratory A	Laboratory B	Foss Winescan
n	100	100	100
Mean	0.85	0.72	0.58
Min	0.21	0.23	0.12
Max	3	4.17	3.27

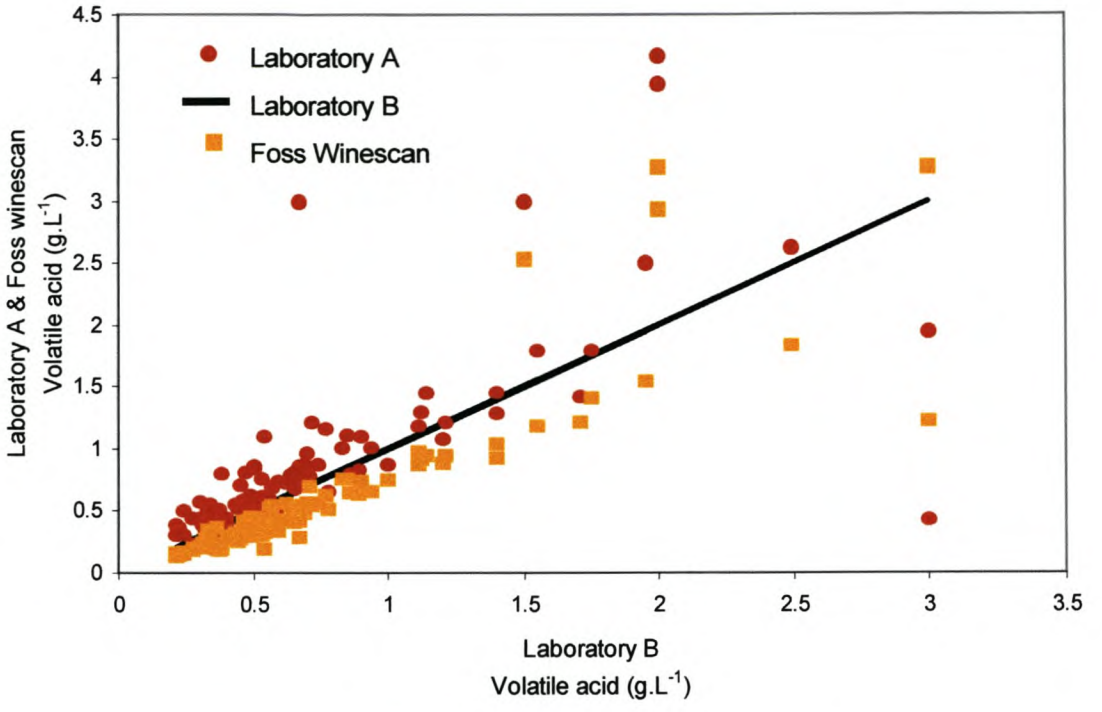

Figure 3. Correlation graph of volatile acid concentrations in the 100 distilling wine samples as measured by two independent laboratories and an instrumental method

Table 5. Analysis of variance for the volatile acidity determinations in distilling wine .

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	p
Laboratories	2	3.744	1.872	20.58	<0.001
Error	198	18.005	0.091		

Table 6. Probabilities obtained from post-hoc analysis (Bonferroni test) performed on the results obtained for volatile acid measurements.

	Laboratory A	Laboratory B	Foss Winescan
Laboratory A		0.006	<0.001
Laboratory B	0.006		0.004
Foss Winescan	<0.001	0.004	

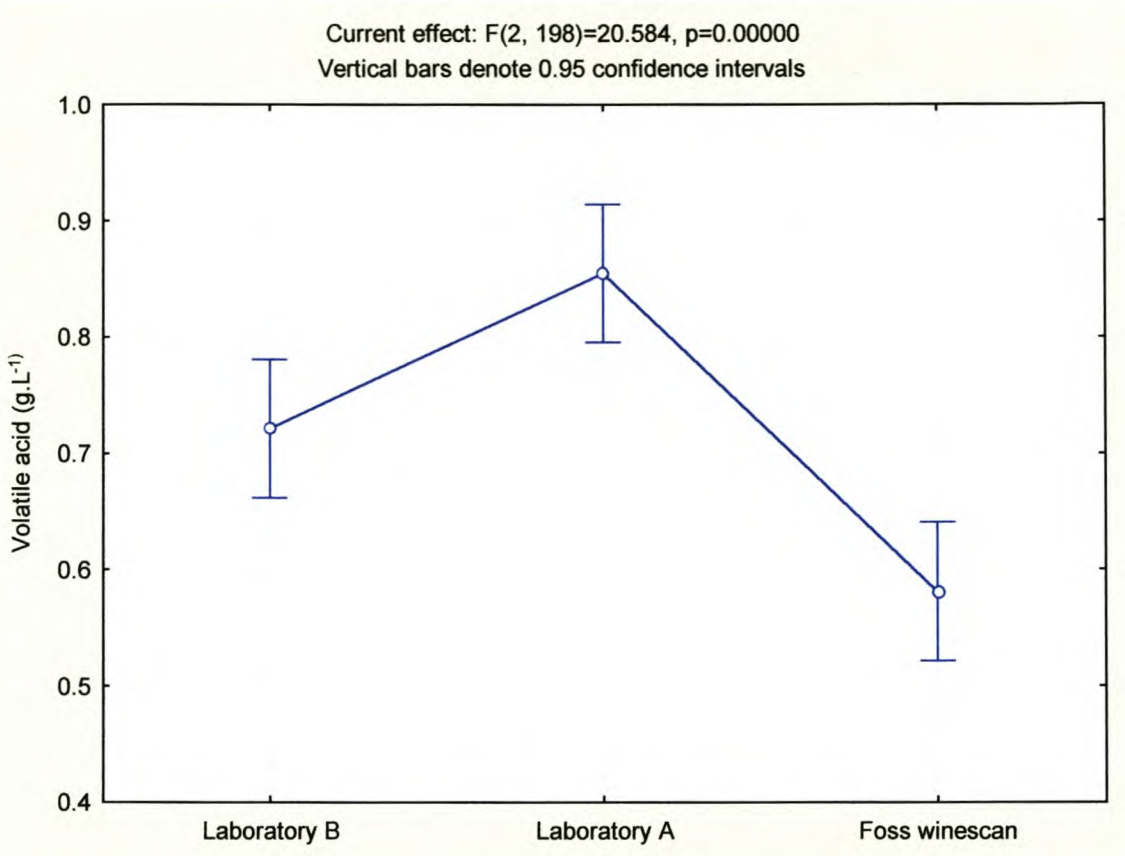

Figure 4. ANOVA plot of the measurement differences obtained for volatile acid concentrations in distilling wine by two independent laboratories and an instrumental method.

Total sulphur dioxide

The values obtained for the measurement of the total sulphur dioxide in 100 wine samples by two independent parties, are shown in Table 7. The correlation plot in Figure 5 show the vast difference between the values obtained from the two laboratories. The measurements performed by laboratory B were generally much lower than the values obtained from laboratory A. The difference between the mean values exceeded 50 $mg.L^{-1}$, which is 10 fold larger than the accepted error of laboratory for the method. The difference between the two sets of references, obtained from a paired t-test, differed very highly significantly ($p<0.001$) as indicated in Table 8. The Box and whisker plot in Figure 6 also showed the large differences between the range and means of the two datasets and concluded that the two laboratories and/or chemical methods employed, differed significantly in their execution of total sulphur dioxide concentration measurements in distilling wine. Laboratory A employed the Ripper method to determine the total sulphur dioxide

Table 7. Results obtained for the measurement of total sulphur dioxide ($mg.L^{-1}$) in distilling wine by two independent laboratories.

	Laboratory A	Laboratory B
n	100	100
Mean	62.37	11.51
Min	39	0
Max	125	80
Standard deviation	17.69	17.04

Table 8. Paired t-test performed on total sulphur dioxide values obtained from two independent laboratories.

Variable	Mean difference	Standard deviation difference	Degrees of freedom	t	p
Laboratories	50.86	17.37	99	-29.28	<0.001

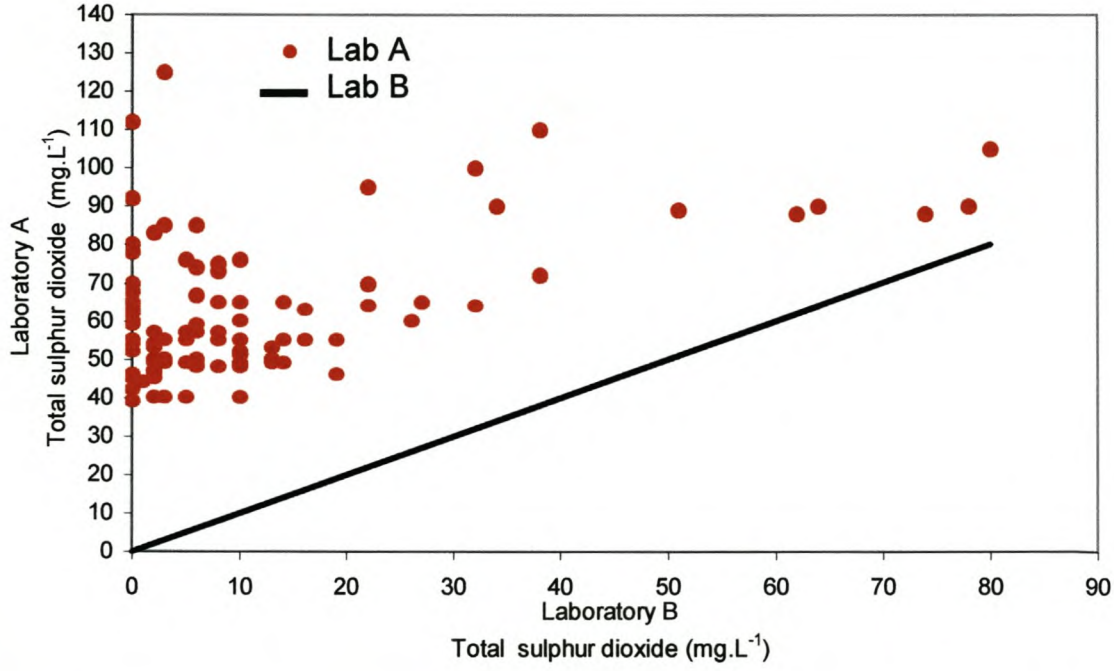

Figure 5. Correlation graph of total sulphur dioxide concentrations in distilling wine as measured by two independent laboratories.

levels in the distilling wine samples. The Ripper method for sulphur dioxide, which is more than 100 years old, employs standard iodine to titrate the total SO_2 in a sample. The method is extremely simple to perform, but universally recognised to be rather inaccurate.

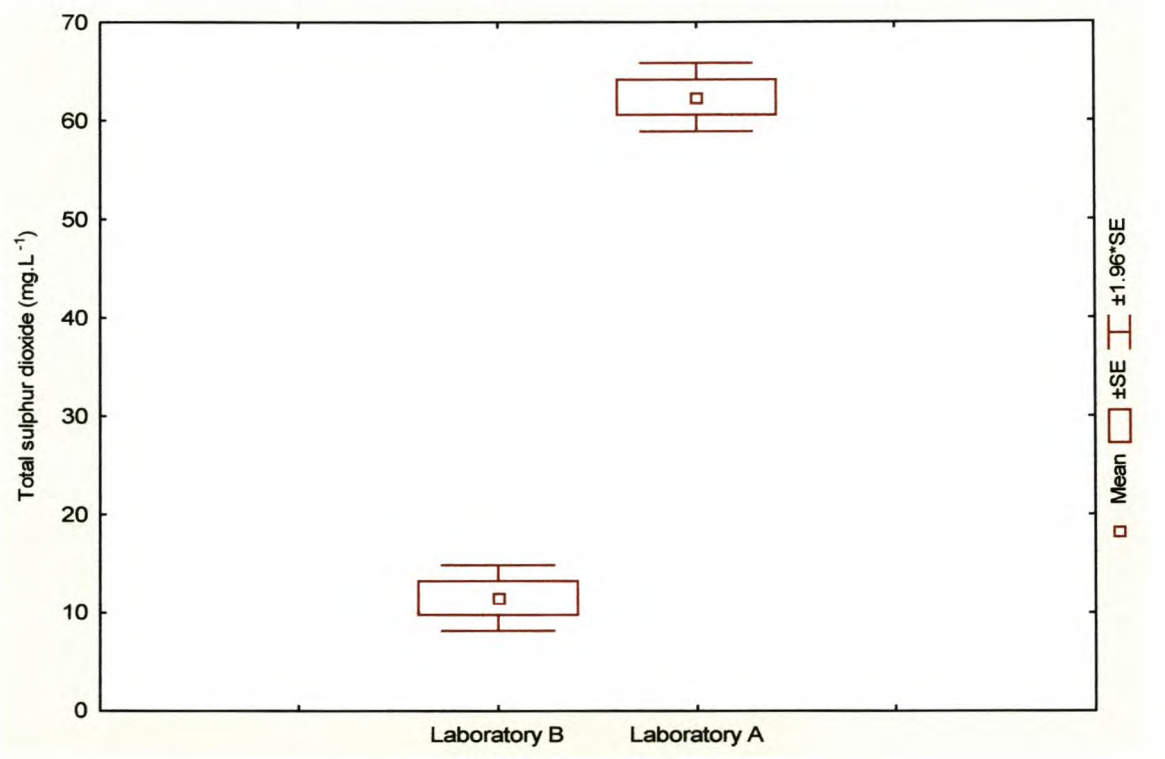

Figure 6. Box and whisker plot of the range of the total sulphur dioxide content of distilling wine as measured by two independent laboratories.

Conclusions

Neither the alcohol nor the volatile acid concentrations were precise or well correlated amongst the three measurements. A very poor correlation was found between the two laboratories for the total sulphur dioxide measurements. The reasons for the unsatisfactory results need to be further investigated, and sources of error for each method of analysis must be determined and eliminated. The results of this small and limited study do suggest that a need exists for a laboratory proficiency survey amongst wineries, producers and independent laboratories in the Western Cape. The SEL of the measurements should be determined by blind duplicate. A preliminary study incorporating 30 or more wineries from the Stellenbosch area with requested information about 10 or more common wine analyses (e.g. pH, titratable

acid, residual sugar, alcohol, volatile acid, sulphur dioxide, soluble solids, phenolics, carbon dioxide, potassium) could evaluate the analytical situation and probably set the tone for further investigation.

The Foss Winescan FT 120 was supplied with a calibration that was developed on European wines. It is important to note that wines from the Southern hemisphere differ in various respects from wines from the colder Northern hemisphere. South African wines generally have lower acidity and higher pH than wines from Europe. This could have a significant effect on the prediction performance of the analyser with pre-standardised settings and calibrations. The wines were analysed within a short time from each other, but temperature fluctuations could have played a significant role on the composition of the wine, as the study was conducted during the warm summer months of March and April.

The danger of using reference data with such great uncertainty as shown in the study, could have a detrimental effect on NIR calibrations and the reputability of NIRS as an alternative analytical method.

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CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

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GENERAL DISCUSSION AND CONCLUSIONS

Due to strict regulatory demands, both locally and internationally, the wine and distillation industry rely heavily on chemical and automated analyses of various compounds. Whether these routine analyses are performed for qualitative purposes, purely to monitor the advancement of the production process or to monitor the levels of compounds that are defined by legislation, methods that are reliable, rapid and easy to perform will enjoy preference to those that are tedious and complicated to perform. Another great concern to the manufacturing sector nowadays is the generation of waste material, whether through the production processes or the quality control laboratories.

Near infrared spectroscopy (NIRS) could be a possible solution regarding some of these issues in the wine and distillation industry. Distilling wine and brandy base wine are the most important raw materials used during the distillation process of neutral grape spirit and brandy, respectively. Distilling wine is a crude, turbid product whilst brandy base wine is a specific style of wine with strict quality requirements. Certain compounds in brandy base wine have a significant influence on the quality of the brandy that will be distilled from it and, therefore, need to be controlled. These wines must conform to certain specifications to ensure an effective and stable process and a product of consistent quality. Alcohol is the main constituent of both grape spirit and brandy, and is accumulated through the distillation process. The distillation yield can only be satisfactory, therefore, if the alcoholic strength of the wine comply with standards set by the distilleries.

The measurement of alcohol in wine and alcoholic beverages using NIRS has been well established (Kaffka & Norris, 1976; Baumgarten, 1987; Sneyd *et al.*, 1989; Garcia-Jares & Médina, 1997; Van den Berg *et al.*, 1997). The OH first overtone band in alcohols and phenols occurs in the region of 1405-1425 nm with the second overtone between 945 and 985 nm (Osborne *et* al., 1993). The absorption of OH bonds are strong in the near infrared

region, making it a very suitable application for quantitative alcohol determination. In this study, good predictions were obtained for the alcohol concentration of brandy base wine samples (r = 0.92, SECV = 0.18% v/v) and distilling wine samples (r = 0.99, SEP = 0.18% v/v).

Overtone bands of the carboxyl group found in carboxylic acids (organic acids) have been observed in the region 1900 nm (Osborne *et al.*, 1993). Kaffka & Norris (1976) determined the tartaric acid content in wine using selected interference filters. The total and volatile acid levels and the pH in wine, have been predicted successfully with mid-infrared spectroscopy using the Foss Winescan FT 120 (Foss Electric, Denmark) by Gishen & Holdstock (2000). In this study, strong correlations were found between the spectral data and the total acid content (r = 0.89, SECV = 0.38 $g.L^{-1}$), volatile acidity (r = 0.85, SEP = 0.04 $g.L^{-1}$) and total phenolic content (r = 0.71, SECV = 16.4 $mg.L^{-1}$ GAE) in brandy base wine as well as for the pH measurements (r = 0.84, SEP = 0.09). The errors obtained for the prediction of these compounds with the NIRS models, all compared well to the laboratory errors of the relevant reference methods, but the RER values indicated that the accuracy of the predictions in terms of the concentration range of constituents, were not adequate for analytical purposes. Attention should be given to the reference method accuracy and precision to obtain optimal NIRS prediction results.

In contrast to the brandy base wine, the distilling wine yielded unsatisfactory prediction results for the volatile acidity of the wine (r = 0.67, SEP of 0.33 $g.L^{-1}$). The turbidity of the wine samples resulted in noisy data and had a detrimental effect on the regression of the concentration data to the spectral data. Unsatisfactory predictions were obtained for the total sulphur dioxide contents of the distilling wine and the residual sugar and acetaldehyde concentrations of the brandy base wine. The application of NIRS for the determination of residual sugar (0-10 $g.L^{-1}$) has been limited due to lack of success in obtaining reliable calibrations (Gishen & Dambergs, 1998). The second overtone at 1960 nm in aldehydes are probably too weak and too close to the water band at 1940 nm, to be of analytical use in a complex matrix like wine (Osborne *et al.*, 1993). Even though satisfactory correlations have been obtained between infrared spectra and the total sulphur dioxide

concentrations in mixed wine samples (measured with the Foss Winescan FT 120), the predictions errors obtained in the study were too high for future measurement of the total sulphur dioxide contents of wine (Gishen & Holdstock, 2000).

Sample presentation is a very important aspect of NIRS and sample thickness plays an important role when conducting spectral-transmittance measurements (Kawano, 2002). In this study, better prediction results were obtained for the alcohol and volatile acid contents of distilling wine with the spectral measurements performed in a 1 mm quartz cuvette as opposed to a 0.2 mm cuvette.

A different type of calibration problem, where the aim is to classify rather than measure, involves the use of NIRS to distinguish between samples whose composition varies over a very narrow range (Osborne *et al.*, 1993). In this study, SIMCA (soft independent modelling of class analogy) was applied successfully to develop discriminative models to classify unblended three-year old brandy in suitable classes for blending purposes. SIMCA, where each sample type is modelled independently of the others and new samples are treated separately by each cluster model, is reported to have advantages in the separation of very similar materials (Downey & Beauchêne, 1997).

Subjective sensorial classification data served as the reference data for the development of the models, positive predictions were obtained for the spectral data obtained with two different types of near infrared instrumentation i.e. a Fourier-transform near infrared system and a Diode Array UV/Visible spectrophotometer. The brandy classes had to be divided into seasonal datasets to obtain satisfactory discrimination, indicating that seasonal variation plays a major role in near infrared spectra. NIRS as a classification technique, could be of great help to the distilling industry where vast amounts of unblended brandy is tasted frequently by a trained panel to select suitable styles of unblended brandy for specific styles of commercial brandies. The application of this technique could speed-up the selection process and result in a more consistent, objective evaluation process.

The near infrared spectrum contains information about the major organic chemical bonds in products. The spectra generated through NIRS

absorbance measurements are dependent on all the functional groups that absorb near infrared radiation, which in turn are correlated to the major chemical, physical, and or sensory components of a substance (Shenk *et al*, 1992). Furthermore, the spectra also contain information due to light interaction with the sample as well as instrumental, data collection and computational errors. The successful correlation of chemical data to NIRS absorbance measurements is therefore fundamentally dependent on accurate and precise values for the constituents of interest. Laboratory procedures, which are used to calibrate near infrared instruments, are not well defined chemically and can be very difficult to relate to spectroscopic data (Shenk *et al.*, 1992). Proficiency testing is an effective way to evaluate laboratory precision and accuracy, and should serve as a means to improve laboratory efficiency (Lawn *et al.,* 1997). In this study it was found that two independent laboratories and an instrumental method (the Foss Winescan FT 120 with volatile acid and alcohol calibrations) differed significantly from each other ($p<0.05$) in their measurement of the volatile acid contents of the same distilling wine samples. One laboratory differed significantly in its measurement of the alcohol strength of distilling wine from two other references whilst for total sulphur dioxide measurements in distilling wine, very significant differences were obtained between the measurements of two laboratories.

Expensive, time-consuming, labour intensive and waste-generating analytical methods are being used to a great extent by the distillation industry to monitor the quality parameters of raw materials and control certain processes. NIRS could successfully be applied to monitor some of these parameters, especially as a screening method where accuracy and precision of measurement is not as important, but emphasis is placed on speed and detection limit.

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